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CLINICAL PREDICTION MODELS FOR IMMUNOTHERAPY BENEFIT IN ADVANCED SOLID TUMORS: CURRENT EVIDENCE, CHALLENGES, AND FUTURE DIRECTIONS IN LOW-RESOURCE SETTINGS

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ABSTRACT

Background: Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of advanced solid tumours by producing durable responses and improving survival across multiple malignancies. However, only a subset of patients derives sustained clinical benefit, while many experience primary or acquired resistance. Reliable predictive biomarkers are therefore essential to optimize patient selection, minimize unnecessary toxicity, and improve the cost-effectiveness of immunotherapy. Although several biomarkers have been investigated, their predictive performance varies considerably across tumour types, and no single biomarker has demonstrated universal clinical applicability. **Aim**: To systematically review and critically appraise the evidence published between January 2016 and June 2026 regarding clinical, pathological, molecular, immunological, blood-based, and imaging biomarkers associated with response to immune checkpoint inhibitor therapy in patients with advanced solid tumours. **Methods**: A systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines. Electronic databases including PubMed/MEDLINE, Embase, Scopus, Web of Science, Cochrane Library, and ClinicalTrials.gov were searched for studies published between January 2016 and June 2026. Eligible studies included randomized controlled trials, prospective and retrospective cohort studies, and observational studies evaluating predictive biomarkers of immunotherapy response in adults with advanced solid tumours. Two reviewers independently screened studies, extracted data, and assessed methodological quality using validated risk-of-bias tools appropriate to study design. **Results**: A total of 8,200 records were identified through database searching. After removal of duplicates and application of predefined eligibility criteria, 211 studies were included in the qualitative synthesis. Evidence demonstrated that programmed death-ligand 1 (PD-L1) expression remains the most widely used predictive biomarker in routine clinical practice, although its predictive accuracy is limited by biological heterogeneity, assay variability, and inconsistent scoring methods. Microsatellite instability-high (MSI-H) and deficient mismatch repair (dMMR) emerged as the most robust validated biomarkers, consistently predicting durable responses across multiple tumour types. Tumour mutational burden (TMB) provided complementary predictive information but was limited by lack of assay standardization and variable cut-off values. Among emerging biomarkers, circulating tumour DNA (ctDNA) kinetics showed the strongest evidence as a dynamic predictor of treatment response, with early ctDNA clearance correlating with improved objective response rate, progression-free survival, and overall survival. Tumour-infiltrating lymphocytes, interferon-γ-related gene-expression signatures, inflammatory biomarkers including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), lactate dehydrogenase (LDH), and the Lung Immune Prognostic Index (LIPI), together with radiomics and artificial intelligence (AI)-based prediction models, demonstrated promising but variable predictive value. Overall, composite biomarker strategies integrating clinical, molecular, and immunological variables consistently outperformed single biomarkers. **Discussion:** The findings indicate that immunotherapy response is determined by complex interactions between tumour biology, the immune microenvironment, host inflammatory status, and dynamic treatment-related changes. Although PD-L1, MSI-H/dMMR, and TMB remain important components of current clinical decision-making, emerging biomarkers—particularly ctDNA—offer significant opportunities to improve treatment prediction. Integration of multiple complementary biomarkers is likely to represent the future of precision immuno-oncology. In low- and middle-income countries (LMICs), incorporation of affordable biomarkers such as NLR, LDH, PLR, and LIPI alongside selected molecular testing may provide a pragmatic and cost-effective approach to patient selection. **Conclusion:** No single biomarker accurately predicts response to immune checkpoint inhibitor therapy across all advanced solid tumours. Current evidence supports the use of integrated, multimodal biomarker strategies that combine clinical, pathological, molecular, blood-based, and imaging parameters to optimize patient selection. Future research should focus on prospective validation of composite prediction models, standardization of emerging biomarker assays, and development of resource-stratified approaches applicable across diverse healthcare settings.

KEYWORDS: Immunotherapy; Immune checkpoint inhibitors; Predictive biomarkers; PD-L1; Tumour mutational burden; Microsatellite instability; Circulating tumour DNA; Precision oncology; Solid tumours; Systematic review; PRISMA 2020.

INTRODUCTION

Introduction Cancer remains one of the leading causes of morbidity and mortality worldwide, accounting for nearly 20 million new cases and approximately 10 million deaths annually. The global burden is expected to rise substantially over the coming decades because of population growth, ageing, urbanization, and increasing exposure to modifiable risk factors.[1] More than 70% of cancer-related deaths occur in low- and middle-income countries (LMICs), where healthcare systems frequently face limitations in diagnostic capacity, access to treatment, and supportive care. These disparities contribute to poorer outcomes and underscore the need for cost-effective, evidence-based strategies that maximize the benefit of available therapies.

The advent of immune checkpoint inhibitors (ICIs) has transformed the management of advanced solid tumors. Monoclonal antibodies targeting programmed cell death protein 1 (PD-1), programmed death ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) have demonstrated durable clinical responses across a broad spectrum of malignancies, including melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma, urothelial carcinoma, hepatocellular carcinoma, head and neck squamous cell carcinoma, gastric cancer, esophageal cancer, and triple-negative breast cancer. Compared with conventional cytotoxic chemotherapy, ICIs can produce long-term disease control in a subset of patients, fundamentally changing treatment paradigms in oncology.[2-4]

Despite these advances, only a minority of patients derive meaningful benefit from immunotherapy. Objective response rates vary widely according to tumor type, disease stage, treatment regimen, and host factors, while many patients experience primary resistance, early disease progression, or immune-related adverse events. Because ICIs are associated with substantial financial costs and unique toxicities, identifying patients who are most likely to benefit has become a major priority in precision oncology.[5-6]

Several predictive biomarkers have been introduced into clinical practice. PD-L1 expression, assessed by immunohistochemistry, is the most widely used biomarker for treatment selection in several malignancies, although its predictive performance is inconsistent because of intratumoral heterogeneity, assay variability, and dynamic changes in expression over time. Tumor mutational burden (TMB), microsatellite instability (MSI), mismatch repair deficiency (dMMR), gene-expression signatures, circulating tumor DNA (ctDNA), tumor-infiltrating lymphocytes, and radiomic features have also been investigated. While these biomarkers provide valuable biological insights, many require sophisticated laboratory infrastructure, standardized testing platforms, and substantial financial resources, limiting their availability in resource-constrained settings.[6-7]

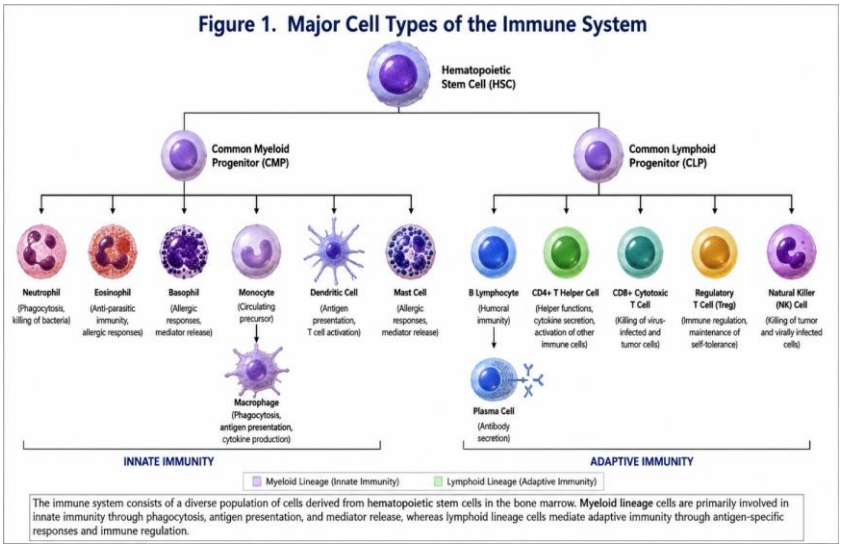
To improve individualized treatment decisions, investigators have developed numerous clinical prediction models that integrate multiple patient-, tumor-, laboratory-, and treatment-related variables. These models include prognostic scores, nomograms, machine-learning algorithms, and multivariable risk calculators designed to estimate the likelihood of response, progression-free survival, overall survival, or durable clinical benefit following immunotherapy. Common predictors include age, Eastern Cooperative Oncology Group (ECOG) performance status, smoking history, body mass index, metastatic burden, neutrophil-to-lymphocyte ratio (NLR), lactate dehydrogenase (LDH), serum albumin, hemoglobin concentration, PD-L1 expression, and molecular biomarkers. By combining several predictors rather than relying on a single biomarker, these models may improve discrimination and support more personalized clinical decision-making.

However, the quality and clinical applicability of published prediction models vary considerably. Many models have been developed using retrospective single-center cohorts, small sample sizes, heterogeneous patient populations, or limited validation. External validation is frequently lacking, calibration is inconsistently reported, and methodological shortcomings may increase the risk of bias. Guidance such as the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) statement, the Prediction Model Risk of Bias Assessment Tool (PROBAST), and the Checklist for Critical Appraisal and Data Extraction for Systematic Reviews of Prediction Modelling Studies (CHARMS) provides a framework for evaluating these studies, yet relatively few reviews have synthesized the evidence using these standards.

The challenges are particularly pronounced in LMICs. Access to comprehensive molecular profiling, next-generation sequencing, TMB assessment, multiplex immunohistochemistry, and advanced imaging biomarkers is often limited by financial, technical, and workforce constraints. Even PD-L1 testing may not be routinely available in many settings. Consequently, prediction models that depend heavily on expensive molecular diagnostics may have limited real-world utility in these regions. In contrast, models incorporating routinely available clinical and laboratory variables could provide practical, affordable tools for treatment stratification and resource allocation.

A systematic synthesis of existing prediction models is therefore needed to identify robust predictors of immunotherapy benefit, compare model performance, evaluate methodological quality, and determine which approaches are feasible across different healthcare settings. Such a review may also identify evidence gaps, including the underrepresentation of LMIC populations, limited external validation, and inconsistent reporting of model performance. These findings can inform future research and facilitate the development of practical prediction strategies tailored to varying levels of healthcare resources.

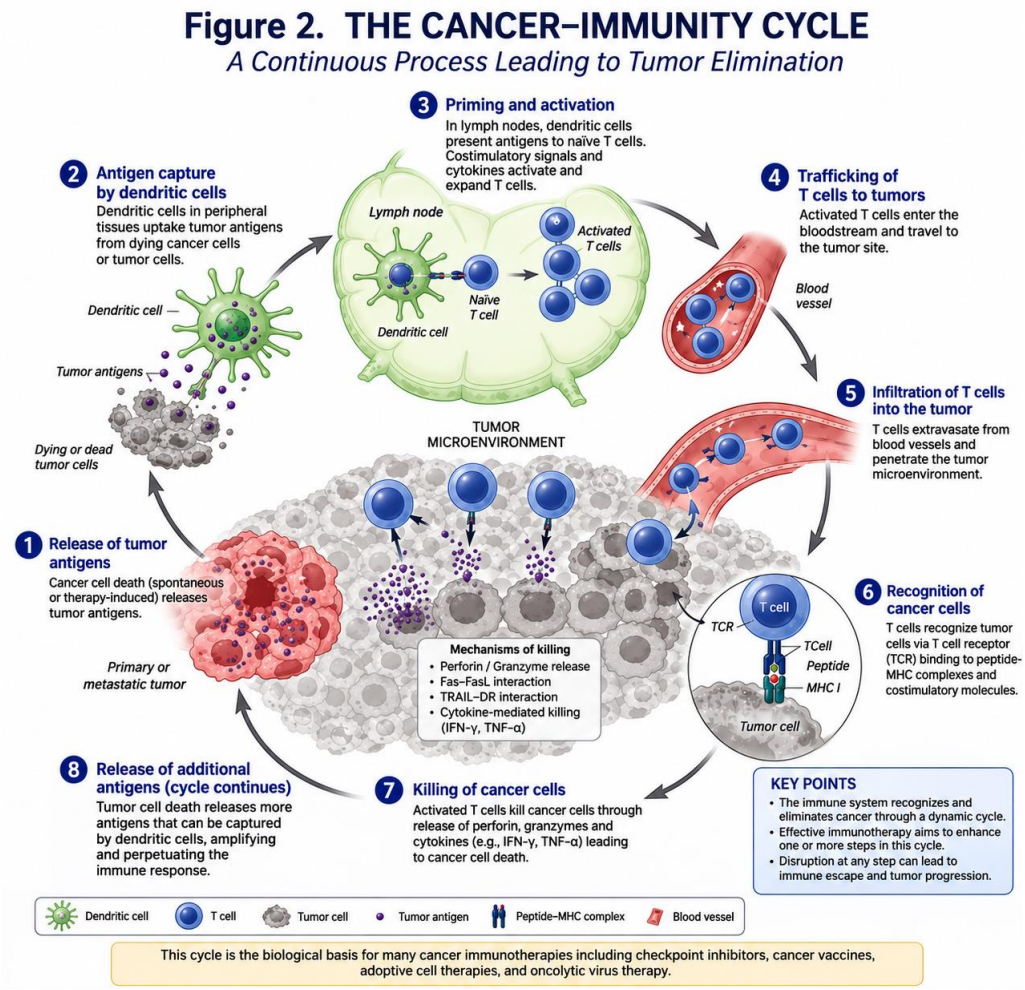
Accordingly, this systematic review aims to identify and critically appraise clinical prediction models developed to predict benefit from immune checkpoint inhibitors in adults with advanced solid tumors. In addition to summarizing model characteristics and performance, the review will evaluate methodological quality using established prediction-model appraisal frameworks and propose a resource-stratified framework to guide implementation in low- and middle-income countries. By integrating evidence from diverse tumor types and considering differences in healthcare resources, this review seeks to contribute to equitable, evidence-based precision oncology.



Chapter 2: Literature Review

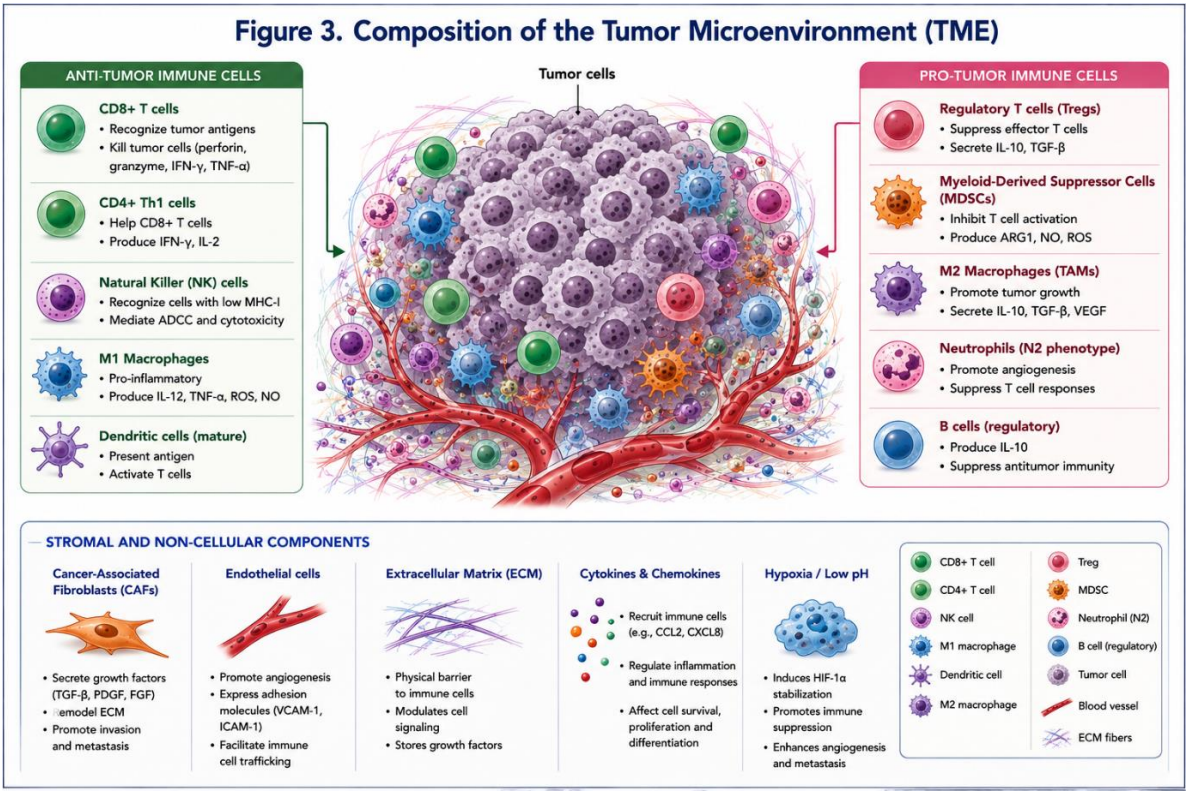
2.1 Evolution of Cancer Immunotherapy

The concept of harnessing the immune system to eliminate malignant cells has evolved over more than a century. Early observations by William Coley suggested that immune activation could induce tumor regression, although the mechanisms remained poorly understood. Advances in tumor immunology eventually led to the identification of immune checkpoints, inhibitory pathways that maintain immune homeostasis by preventing excessive T-cell activation. While these pathways are essential for preventing autoimmunity, many cancers exploit them to evade immune surveillance.



The discovery of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1), together with its ligand PD-L1, revolutionized cancer therapy. Monoclonal antibodies targeting these checkpoints restore antitumor immunity by reactivating exhausted T cells within the tumor microenvironment. Since the approval of ipilimumab for metastatic melanoma in 2011, immune checkpoint inhibitors (ICIs) have become integral to the management of numerous advanced solid tumors.

Today, ICIs are approved for melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma, urothelial carcinoma, hepatocellular carcinoma, head and neck squamous cell carcinoma, gastric cancer, esophageal cancer, cervical cancer, and triple-negative breast cancer, among others. These therapies have demonstrated durable responses and prolonged survival in selected patients, fundamentally changing expectations for metastatic disease.



Nevertheless, objective response rates remain modest in many cancers, and only a subset of patients achieves long-term benefit. Primary resistance, acquired resistance, immune-related adverse events, and the high cost of treatment have intensified the search for reliable methods to identify patients most likely to benefit.

Table 1. Major Cancer Immunotherapy Approaches

Immunotherapy Approach	Mechanism of Action	Representative Agents	Approved Cancer Types (Examples)	Key Advantages	Major Limitations
1. Immune Checkpoint Inhibitors (ICIs)	Block inhibitory immune checkpoints (PD-1, PD-L1, CTLA-4) to restore T-cell activity against tumour cells.	• Pembrolizumab • Nivolumab • Atezolizumab • Durvalumab • Cemiplimab • Ipilimumab	NSCLC, Melanoma, RCC, Urothelial carcinoma, HNSCC, Gastric cancer, TNBC, HCC	Durable responses; broad clinical applicability.	Immune-related adverse events (irAEs); primary and acquired resistance.
2. CAR-T Cell Therapy	Patient-derived T cells are genetically engineered to express chimeric antigen receptors targeting tumour antigens.	• Tisagenlecleucel • Axicabtagene ciloleucel • Lisocabtagene maraleucel	B-cell ALL, Diffuse Large B-cell Lymphoma, Mantle Cell Lymphoma, Multiple Myeloma	High response rates in hematological malignancies.	High cost, cytokine release syndrome (CRS), neurotoxicity, limited efficacy in solid tumours.
3. Cancer Vaccines	Stimulate adaptive immune responses against tumour-specific or tumour-associated antigens.	• Sipuleucel-T • Talimogene Laherparepvec (T-VEC) • Personalized neoantigen vaccines	Prostate cancer, Melanoma, Clinical trials in multiple solid tumours	Antigen-specific immunity with favorable safety profile.	Limited efficacy as monotherapy; individual-ized vaccine production.
4. Oncolytic Virus Therapy	Selectively infects and lyses tumour cells while stimulating antitumour immune responses.	• Talimogene Laherparepvec (T-VEC) • Oncorine (China)	Melanoma, Investigational in other solid tumours	Direct tumour lysis with immune activation.	Mostly effective for injectable lesions; limited systemic efficacy.
5. Adoptive Cell Therapy (ACT)	Expansion and reinfusion of tumour-reactive lymphocytes or genetically modified immune cells.	• Tumour-Infiltrating Lymphocytes (TILs) • TCR-engineered T cells	Melanoma, Cervical cancer, Investigational in various solid tumours	Personalized treatment with durable responses.	Complex manufacturing; requires specialized centres.
6. Monoclonal Antibodies	Target tumour-associated antigens, induce ADCC, complement activation, or block growth factor signalling.	• Rituximab • Trastuzumab • Cetuximab • Daratumumab	Breast cancer, Lymphomas, Colorectal cancer, Multiple Myeloma	Established efficacy; often combined with chemotherapy.	Antigen escape, infusion reactions, resistance.
7. Cytokine Therapy	Enhances immune-cell proliferation and activation using recombinant cytokines.	• Interleukin-2 (IL-2) • Interferon-α	Melanoma, Renal Cell Carcinoma	Durable responses in selected patients.	Significant systemic toxicity; limited current use.
8. Bispecific Antibodies / T-cell Engagers	Simultaneously bind tumour antigens and CD3 on T cells to facilitate targeted cytotoxicity.	• Blinatumomab • Tebentafusp • Emerging bispecific antibodies	B-cell ALL, Uveal melanoma, Clinical trials in solid tumours	Off-the-shelf immune redirection.	Cytokine release syndrome; limited approved indications.
9. Combination Immunotherapy	Combines ICIs with chemotherapy, targeted therapy, radiotherapy, or dual checkpoint blockade.	• Nivolumab + Ipilimumab • Pembrolizumab + Chemotherapy	Multiple advanced solid tumours	Improved response rates and survival.	Increased toxicity and treatment cost.

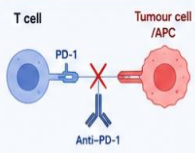
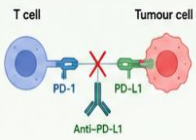
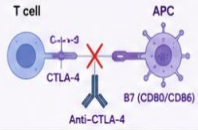
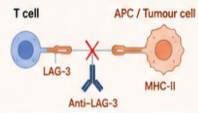
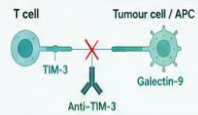
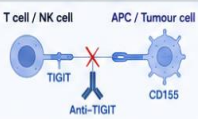
Abbreviations: ALL = Acute Lymphoblastic Leukemia; CAR-T = Chimeric Antigen Receptor T-cell; HCC = Hepatocellular Carcinoma; HNSCC = Head and Neck Squamous Cell Carcinoma; ICI = Immune Checkpoint Inhibitor; IL-2 = Interleukin-2; NSCLC = Non-Small Cell Lung Cancer; RCC = Renal Cell Carcinoma; TCR = T-cell Receptor; TIL = Tumour-Infiltrating Lymphocyte; TNBC = Triple-Negative Breast Cancer.

2.2 Mechanisms of Immune Checkpoint Inhibitors

Tumor cells evade immune destruction through several mechanisms, including reduced antigen presentation, secretion of immunosuppressive cytokines, recruitment of regulatory immune cells, and activation of inhibitory checkpoint pathways. The PD-1/PD-L1 axis suppresses cytotoxic T-cell function after PD-1 on activated lymphocytes binds PD-L1 expressed on tumor cells or immune cells within the tumor microenvironment. CTLA-4 primarily regulates T-cell activation during antigen presentation within lymphoid tissues.

Immune checkpoint inhibitors interrupt these inhibitory signals, enhancing T-cell proliferation, cytokine production, and cytotoxic activity. Combination therapy targeting both CTLA-4 and PD-1 may produce greater immune activation but also increases immune-related toxicity. The effectiveness of ICIs depends on a complex interaction among tumor genomics, host immunity, the tumor microenvironment, microbiome composition, and clinical factors such as performance status and systemic inflammation.

Table 2. Overview of Immune Checkpoint Inhibitors

Checkpoint (Target)	Target / Pathway (Schematic)	Mechanism of Action	Approved Agents (Examples)	Approved Indications (Examples)	Key Adverse Effects	Key Features
PD-1 Inhibitors (PD-1)		Blockade of PD-1 on T cells prevents its interaction with PD-L1/ PD-L2, restoring T-cell activation and antitumour immune response.	• Pembrolizumab • Nivolumab • Cemiplimab	• NSCLC • Melanoma • RCC • HNSCC • Hodgkin lymphoma • MSI-H/dMMR tumours • Others	• Fatigue • Rash • Diarrhoea/colitis • Hepatitis • Pneumonitis • Endocrinopathies	Most widely used ICIs; durable responses in multiple cancers; efficacy independent of PD-L1 in some indications.
PD-L1 Inhibitors (PD-L1)		Blockade of PD-L1 on tumour cells or APCs prevents binding to PD-1, releasing inhibition of T-cell function.	• Atezolizumab • Durvalumab • Avelumab	• NSCLC • Urothelial carcinoma • HNSCC • Triple-negative breast cancer • Gastric cancer • Others	• Fatigue • Rash • Diarrhoea • Hepatitis • Pneumonitis • Endocrinopathies	May be preferred when PD-L1 expression is high; fewer immune related adverse events in some studies vs PD-1 inhibitors.
CTLA-4 Inhibitors (CTLA-4)		Blockade of CTLA-4 prevents its binding to B7 (CD80/CD86) on APCs, enhancing T-cell priming and proliferation.	• Ipilimumab • Tremelimumab	• Melanoma • RCC • mCRC (MSI-H) • Others (in combination)	• Colitis • Hepatitis • Dermatitis • Endocrinopathies • Hypophysitis	First checkpoint inhibitors approved; more irAEs than PD-1/PD-L1 inhibitors; used in combination for synergy.
LAG-3 Inhibitors (LAG-3)		Blockade of LAG-3 reverses inhibition of T-cell activation and proliferation.	• Relatlimab • Lirilumab • Fianlimab (Investigational)	• Melanoma (in combination) • NSCLC (investigational) • Others (clinical trials)	• Fatigue • Rash • Diarrhoea • Hepatitis	Emerging target; promising in combination with PD-1 inhibitors.
TIM-3 Inhibitors (TIM-3)		Blockade of TIM-3 prevents binding to its ligands (e.g., Galectin-9), restoring T-cell function.	• TSR-022 • Sym023 • MBG453 (Investigational)	• NSCLC (investigational) • HCC (investigational) • Others (clinical trials)	• Fatigue • Rash • Diarrhoea • Elevated liver enzymes	Investigational target; may evercome resistance to PD-1 blockade.
TIGIT Inhibitors (TIGIT)		Blockade of TIGIT interference with co-stimulatory signals (CD155/CD112), enhancing T and NK cell function.	• Tiragolumab • Domvanalimab • Vibostolimab (Investigational)	• NSCLC (investigational) • HCC (investigational) • Others (clinical trials)	• Fatigue • Rash • Diarrhoea • Infusion reactions	Novel co-inhibitory axis; best results in combination with PD-1/PD-L1 inhibitors.

Abbreviations: APC = Antigen Presenting Cell; CTLA-4 = Cytotoxic T-Lymphocyte Associated Protein 4; HCC = Hepatocellular Carcinoma; HNSCC = Head and Neck Squamous Cell Carcinoma; ICI = Immune Checkpoint Inhibitor; MSI-H = Microsatellite Instability-High; mCRC = Metastatic Colorectal Cancer; NK = Natural Killer; NSCLC = Non-Small Cell Lung Cancer; PD-1 = Programmed Cell Death Protein 1; PD-L1 = Programmed Death-Ligand 1; RCC = Renal Cell Carcinoma; irAEs = Immune-related Adverse Events.

2.3 Clinical Challenges in Immunotherapy

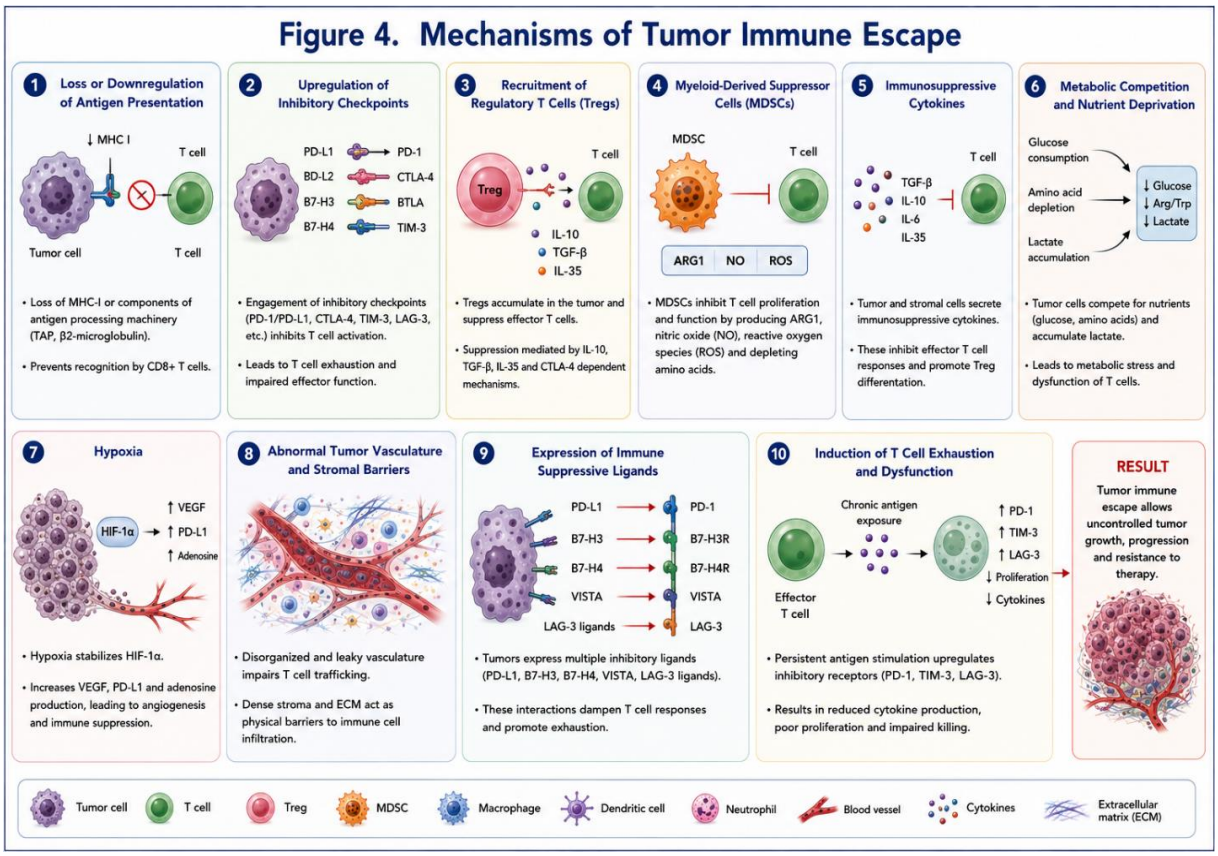
Although ICIs have transformed oncology practice, several important challenges remain.

First, response rates vary substantially across tumor types. Melanoma and mismatch repair-deficient colorectal cancer generally exhibit high response rates, whereas pancreatic cancer and most microsatellite-stable colorectal cancers remain relatively resistant.

Second, reliable biomarkers remain elusive. PD-L1 expression, while widely used, demonstrates imperfect sensitivity and specificity. Some patients with low or absent PD-L1 expression experience durable responses, whereas many PD-L1-positive tumors fail to respond.

Third, immunotherapy is associated with substantial financial costs. Drug acquisition, biomarker testing, toxicity management, and long-term follow-up place considerable strain on healthcare systems, particularly in LMICs.

Fourth, immune-related adverse events affecting the skin, endocrine glands, lungs, gastrointestinal tract, liver, kidneys, and nervous system require specialized multidisciplinary management.



These challenges have encouraged investigators to develop multivariable prediction models capable of integrating multiple sources of clinical information rather than relying on a single biomarker.

2.4 Biomarkers of Immunotherapy Response PD-L1 Expression PD-L1 immunohistochemistry remains the most widely used predictive biomarker in routine clinical practice. Higher PD-L1 expression is generally associated with improved response to PD-1 or PD-L1 blockade in several cancers, particularly NSCLC. However, assay heterogeneity, differing antibody clones, variable scoring systems, temporal variation, and intratumoral heterogeneity limit its reproducibility and predictive accuracy.

Tumor Mutational Burden Tumor mutational burden (TMB) estimates the number of somatic mutations within a tumor genome. Tumors with high mutation burdens generate more neoantigens, potentially increasing immune recognition and response to ICIs. Despite its biological rationale, TMB assessment requires next-generation sequencing, lacks standardized cut-off values across tumor types, and remains expensive for routine use in many healthcare settings.

Microsatellite Instability and Mismatch Repair Deficiency Microsatellite instability-high (MSI-H) and deficient mismatch repair (dMMR) tumors exhibit high mutation burdens and increased neoantigen formation. Pembrolizumab became the first tissue-agnostic cancer therapy approved based on MSI-H/dMMR status. Although highly predictive where present, these alterations occur in only a minority of solid tumors.

Tumor-Infiltrating Lymphocytes The density and spatial distribution of CD8-positive T cells within tumors reflect pre-existing antitumor immunity. Numerous studies have associated increased tumor-infiltrating lymphocytes with improved outcomes following immunotherapy. However, standardized quantification methods remain under development.

Gene-Expression Signatures Gene-expression profiling can identify immune-active tumor microenvironments characterized by interferon signaling, T-cell activation, and inflammatory pathways. These signatures may outperform single biomarkers but require sophisticated molecular platforms and standardized analytical pipelines.

Circulating Biomarkers Peripheral blood biomarkers are attractive because they are inexpensive and minimally invasive. Commonly investigated markers include:
Neutrophil-to-lymphocyte ratio (NLR)
Platelet-to-lymphocyte ratio
Systemic immune-inflammation index
Absolute lymphocyte count
Lactate dehydrogenase (LDH)
Albumin
C-reactive protein
Circulating tumor DNA (ctDNA)

Among these, NLR and LDH have been consistently associated with immunotherapy outcomes and are readily available in routine clinical practice, making them particularly relevant for resource-limited settings.

2.5 Clinical Prediction Models Clinical prediction models combine multiple predictors to estimate an individual's probability of a future clinical outcome. In immuno-oncology, these models aim to predict objective response, progression-free survival, overall survival, durable clinical benefit, or the risk of severe immune-related adverse events. Models have been developed using conventional multivariable regression, nomograms, machine-learning algorithms, and artificial intelligence approaches. Common predictors include demographic variables, ECOG performance status, metastatic burden, laboratory markers, PD-L1 expression, genomic alterations, radiomic features, and treatment characteristics.

Compared with single biomarkers, multivariable models better reflect the multifactorial nature of immunotherapy response. Nevertheless, model performance varies considerably because of differences in study design, patient populations, predictor selection, outcome definitions, and validation strategies.

2.6 Methodological Considerations in Prediction Model Research

High-quality prediction models require adequate sample sizes, careful predictor selection, appropriate handling of missing data, internal validation, external validation, calibration assessment, and transparent reporting. Unfortunately, many published models suffer from methodological limitations, including retrospective study designs, small cohorts, overfitting, and inadequate validation.

The Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) statement provides guidance for complete reporting of prediction model studies. The Prediction Model Risk of Bias Assessment Tool (PROBAST) evaluates risk of bias across participant selection, predictors, outcomes, and statistical analysis. The CHARMS checklist supports standardized data extraction for systematic reviews of prediction models. Adherence to these frameworks improves reproducibility and facilitates comparisons across studies.

2.7 Artificial Intelligence and Machine Learning

Recent years have witnessed increasing application of artificial intelligence (AI) and machine learning (ML) in immunotherapy prediction. Deep learning, random forests, gradient boosting, support vector machines, and neural networks have been used to integrate clinical, genomic, radiologic, and pathological data.

Radiomics extracts quantitative imaging features from CT, MRI, or PET scans, while digital pathology uses whole-slide imaging to characterize the tumor microenvironment. Multi-omics approaches combine genomic, transcriptomic, proteomic, and metabolomic data to improve predictive accuracy.

Despite promising results, AI-based models often lack external validation and may require computational infrastructure that is unavailable in many LMICs.

2.8 Challenges in Low- and Middle-Income Countries

LMICs face significant barriers to implementing precision oncology. These include limited access to molecular diagnostics, shortages of trained personnel, inadequate pathology services, inconsistent availability of PD-L1 testing, restricted access to next-generation sequencing, and high treatment costs.

In many settings, clinicians rely primarily on clinical examination and basic laboratory investigations when making treatment decisions. Consequently, there is a pressing need for prediction models that incorporate inexpensive, routinely available variables such as ECOG performance status, complete blood count, serum albumin, LDH, and metastatic burden.

A resource-stratified framework that aligns prediction tools with available infrastructure may help optimize patient selection while promoting equitable access to immunotherapy.

2.9 Evidence Gap

Although numerous prediction models have been reported across different tumor types, the literature remains fragmented. Existing reviews frequently focus on individual biomarkers such as PD-L1 or TMB, specific malignancies, or AI methodologies. Few reviews comprehensively evaluate multivariable clinical prediction models across advanced solid tumors, assess their methodological quality using PROBAST and TRIPOD, and consider their applicability in resource-constrained healthcare systems.

Furthermore, external validation in LMIC populations is rare, and the feasibility of implementing complex molecular models outside high-income countries has received limited attention. These gaps justify the need for a systematic review that synthesizes the available evidence and proposes a practical, resource-stratified framework for clinical implementation.

Chapter Summary

The literature demonstrates that immune checkpoint inhibitors have transformed the treatment of advanced solid tumors but have also highlighted the need for accurate patient selection. Existing biomarkers each have important limitations, and multivariable prediction models offer a promising strategy to improve individualized treatment decisions. However, methodological heterogeneity, limited external validation, and unequal access to advanced diagnostics reduce the immediate clinical applicability of many published models. A systematic review focusing on prediction models and their implementation in LMICs is therefore warranted and may help bridge the gap between precision oncology research and routine clinical practice.

Chapter 3: Rationale and Justification of the Study

3.1 Rationale

The introduction of immune checkpoint inhibitors (ICIs) has fundamentally transformed the management of advanced solid tumors, providing durable responses and prolonged survival for a subset of patients with malignancies such as non-small cell lung cancer, melanoma, renal cell carcinoma, urothelial carcinoma, hepatocellular carcinoma, head and neck squamous cell carcinoma, gastric cancer, and several other advanced cancers. These therapies have become an integral component of modern oncology and have shifted treatment paradigms from conventional cytotoxic chemotherapy toward precision immuno-oncology.

Despite these advances, only a proportion of patients derive meaningful clinical benefit from immunotherapy. Depending on tumor type and treatment regimen, objective response rates generally range from approximately 15% to 50%, indicating that many patients are exposed to expensive treatment without achieving durable disease control. In addition to primary resistance, some patients experience severe immune-related adverse events that may require prolonged immunosuppressive therapy, hospitalization, or permanent discontinuation of treatment. Consequently, accurately identifying patients who are most likely to benefit from ICIs has become a major priority in contemporary oncology.

Current patient-selection strategies rely heavily on individual biomarkers, particularly programmed death ligand-1 (PD-L1) expression, microsatellite instability (MSI), deficient mismatch repair (dMMR), and tumor mutational burden (TMB). While these biomarkers have improved treatment selection for specific malignancies, each has important limitations. PD-L1 expression demonstrates significant inter-assay variability, intratumoral heterogeneity, and temporal changes during disease progression. TMB requires next-generation sequencing, lacks universally accepted cut-off values across tumor types, and remains costly. MSI-H and dMMR status are highly predictive when present but occur in only a small proportion of advanced solid tumors. Consequently, no single biomarker adequately predicts immunotherapy benefit across all malignancies.

Recognizing these limitations, researchers have increasingly developed multivariable clinical prediction models that combine demographic characteristics, clinical variables, laboratory parameters, pathological findings, molecular

biomarkers, imaging features, and treatment characteristics to estimate the likelihood of response, progression-free survival, overall survival, or durable clinical benefit. These models have been developed using traditional regression methods, nomograms, machine learning algorithms, and artificial intelligence approaches. By integrating multiple sources of information, prediction models may provide more accurate and individualized estimates of treatment benefit than any single biomarker alone.

However, the rapidly expanding literature on immunotherapy prediction models presents several challenges. First, prediction models have been developed across diverse tumor types using heterogeneous study populations, variable outcome definitions, different predictor variables, and inconsistent statistical methods. This heterogeneity complicates direct comparisons between studies and limits clinicians' ability to determine which models are most reliable for routine practice.

Second, many published models have methodological limitations that reduce their credibility and clinical applicability. Common concerns include retrospective study designs, single-center cohorts, relatively small sample sizes, inappropriate variable selection strategies, incomplete handling of missing data, inadequate reporting of calibration, and lack of external validation. These issues increase the risk of overfitting and reduce confidence in the generalizability of reported performance measures. Although internationally recognized methodological standards such as the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) Statement, the Prediction Model Risk of Bias Assessment Tool (PROBAST), and the Checklist for Critical Appraisal and Data Extraction for Systematic Reviews of Prediction Modelling Studies (CHARMS) have been developed to improve the quality of prediction-model research, relatively few evidence syntheses have systematically applied these frameworks across immunotherapy prediction models spanning multiple solid tumor types.

Third, existing reviews have predominantly focused on individual biomarkers or specific malignancies rather than evaluating multivariable clinical prediction models across advanced solid tumors. Reviews of PD-L1, TMB, MSI, gene-expression signatures, radiomics, or artificial intelligence have contributed substantially to understanding the biology of immunotherapy response but do not provide a comprehensive assessment of clinically applicable prediction tools that integrate multiple predictors. As a result, clinicians lack a consolidated evidence base to compare available models, assess methodological quality, and identify those most suitable for implementation.

A particularly important gap concerns the applicability of prediction models in low- and middle-income countries (LMICs). Most prediction models have been developed and validated in high-income countries where advanced molecular diagnostics, next-generation sequencing, digital pathology, and sophisticated imaging technologies are widely available. In contrast, many oncology centers in LMICs continue to face shortages of pathology services, molecular laboratories, trained personnel, and financial resources. Even when immunotherapy is available, routine access to PD-L1 testing, TMB assessment, comprehensive genomic profiling, multiplex immunohistochemistry, or circulating tumor DNA analysis may be limited or absent. Prediction models that depend heavily on expensive molecular investigations may therefore have limited real-world utility in resource-constrained settings.

Conversely, several inexpensive clinical and laboratory variables—including Eastern Cooperative Oncology Group (ECOG) performance status, neutrophil-to-lymphocyte ratio (NLR), lactate dehydrogenase (LDH), serum albumin, hemoglobin concentration, body mass index, smoking history, and metastatic burden—have consistently demonstrated

prognostic or predictive value in patients receiving ICIs. These parameters are routinely measured in most oncology centers worldwide and could form the basis of practical prediction models suitable for implementation across different levels of healthcare infrastructure. However, the evidence supporting these simplified models has not been comprehensively synthesized, and their potential role within resource-stratified clinical pathways remains insufficiently explored.

A systematic review focused specifically on clinical prediction models for immunotherapy benefit is therefore warranted. By systematically identifying, critically appraising, and comparing published models, this review will provide clinicians and researchers with a comprehensive overview of available prediction tools, their methodological quality, validation status, predictive performance, and feasibility for routine clinical use. The review will also identify gaps in the current literature, including underrepresentation of LMIC populations, limited external validation, inconsistent reporting standards, and insufficient evaluation of implementation feasibility.

Importantly, the findings of this review will be used to develop a proposed resource-stratified framework that aligns prediction strategies with available healthcare resources. Such a framework recognizes that not all healthcare systems have access to the same diagnostic technologies and seeks to recommend pragmatic approaches that maximize the use of routinely available clinical information while incorporating advanced biomarkers where feasible. This approach is consistent with international efforts to promote equitable access to precision oncology and optimize resource allocation within constrained healthcare environments.

3.2 Justification of the Study

This systematic review is justified for several reasons.

First, the increasing global use of immune checkpoint inhibitors has created an urgent need for reliable methods to identify patients who are most likely to benefit from treatment. Improved prediction of treatment response has the potential to enhance clinical outcomes while reducing unnecessary toxicity and healthcare expenditure.

Second, no universally accepted clinical prediction model currently exists for predicting immunotherapy benefit across advanced solid tumors. Available models differ substantially in their predictors, statistical methodology, validation, and reported performance. A comprehensive synthesis is required to evaluate their strengths, limitations, and readiness for clinical implementation.

Third, the methodological quality of prediction models has not been consistently evaluated using standardized appraisal frameworks such as PROBAST and TRIPOD. Applying these tools within a systematic review will help distinguish robust models from those at high risk of bias and identify priorities for future model development and validation.

Fourth, the evidence base is dominated by studies from high-income countries. Patients treated in LMICs are underrepresented despite accounting for a large and growing proportion of the global cancer burden. This imbalance limits the generalizability of many published models and highlights the need to consider implementation within diverse healthcare settings.

Finally, the proposed resource-stratified framework represents a practical contribution beyond conventional evidence synthesis. Rather than simply summarizing published studies, the framework will integrate current evidence into recommendations tailored to different levels of diagnostic capacity, ranging from settings that rely primarily on clinical

assessment and routine laboratory testing to centers with access to advanced molecular profiling. Such an approach may support equitable implementation of precision oncology, inform future guideline development, and identify research priorities for validating prediction models in resource-constrained environments.

3.3 Significance of the Study

The findings of this review are expected to benefit several stakeholder groups. For clinicians, the review will provide an evidence-based summary of available prediction models and their potential role in treatment decision-making. For researchers, it will identify methodological limitations, evidence gaps, and priorities for future model development and external validation. For healthcare policymakers and cancer programs, the proposed resource-stratified framework may assist in planning diagnostic services and prioritizing cost-effective approaches to patient selection. Most importantly, by emphasizing models that incorporate routinely available clinical and laboratory variables alongside advanced biomarkers where appropriate, the review seeks to promote more equitable access to precision immunotherapy across healthcare systems with differing levels of resources.

Chapter 4: Study Objectives and Research Questions.

4.1 Aim of the Study

The overall aim of this systematic review is to identify, critically appraise, and synthesize published clinical prediction models developed to predict clinical benefit from immune checkpoint inhibitor therapy in adults with advanced solid tumors and to propose a resource-stratified framework for implementing these models in low- and middle-income countries (LMICs).

4.2 Primary Objective

To systematically identify and critically evaluate published multivariable clinical prediction models designed to predict treatment benefit from immune checkpoint inhibitors in patients with advanced solid tumors.

4.3 Secondary Objectives

The secondary objectives of this systematic review are:

To describe the characteristics of published clinical prediction models, including study design, patient population, cancer type, predictors, statistical methodology, and intended clinical use.

To summarize the predictors incorporated into existing models, including:

Demographic variables

Clinical characteristics

Laboratory biomarkers

Pathological biomarkers

Molecular biomarkers

Radiomic features

Artificial intelligence–derived variables

To compare the predictive performance of published models using reported discrimination and calibration measures, including:

Area under the receiver operating characteristic curve (AUC)

Concordance index (C-index)

Sensitivity

Specificity

Positive predictive value

Negative predictive value

Calibration statistics

Decision-curve analysis where available

To determine the extent of internal and external validation performed for each prediction model.

To evaluate the methodological quality and risk of bias of included studies using the Prediction Model Risk of Bias Assessment Tool (PROBAST).

To assess adherence to the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) reporting guidelines.

To identify methodological limitations and evidence gaps in current immunotherapy prediction-model research.
To evaluate the feasibility of implementing published prediction models in resource-limited healthcare systems.

To develop a practical resource-stratified framework for applying prediction models across basic-, limited-, and enhanced-resource oncology settings.

Table 3. Study Objectives and Outcomes

Objective Type	Study Objective	Primary Outcome(s)	Secondary Outcome(s)
Primary Objective	To systematically evaluate published clinical prediction models for immunotherapy benefit in advanced solid tumours.	Identification of validated prediction models and their predictive performance (AUC/C-index, calibration, discrimination).	• Assessment of methodological quality and clinical applicability.
1 Secondary Objective 1	To identify clinical, pathological, laboratory and molecular predictors associated with immunotherapy response.	Frequency and strength of predictive biomarkers.	• Comparative performance of individual biomarkers across tumour types.
2 Secondary Objective 2	To compare the predictive accuracy of established and emerging biomarkers.	Predictive value of PD-L1, TMB, MSI-H/dMMR, ctDNA and inflammatory biomarkers.	• Emerging evidence for radiomics, gene-expression signatures and AI-based biomarkers.
3 Secondary Objective 3	To evaluate the methodological quality and risk of bias of included studies.	PROBAST risk-of-bias assessment.	• TRIPOD reporting quality. • Overall evidence certainty (GRADE/QUIPS).
4 Secondary Objective 4	To assess the applicability of prediction models in low- and middle-income countries (LMICs).	Feasibility of biomarker-based prediction models in resource-limited settings.	• Cost-effectiveness. • Infrastructure requirements and implementation barriers.
5 Secondary Objective 5	To develop a resource-stratified conceptual framework for immunotherapy prediction.	Proposed integrated prediction framework suitable for LMICs.	• Future research priorities and validation recommendations.

Abbreviations: AUC, area under the curve; ctDNA, circulating tumour DNA; dMMR, deficient mismatch repair; LMICs, low- and middle-income countries; MSI-H, microsatellite instability-high; PD-L1, programmed death-ligand 1; TMB, tumour mutational burden.

4.4 Research Questions

This systematic review seeks to answer the following research questions.

Primary Research Question What clinical prediction models have been developed to predict benefit from immune checkpoint inhibitor therapy in adults with advanced solid tumors, and how well do they perform?

Secondary Research Questions Which predictors are most commonly incorporated into published immunotherapy prediction models?

Which prediction models demonstrate the highest predictive accuracy?

Which models have undergone external validation?

What is the methodological quality of currently available prediction models?

Which prediction models have the lowest risk of bias according to PROBAST?

How completely are prediction-model studies reported according to TRIPOD?

Which predictors are consistently associated with improved immunotherapy outcomes?

Which prediction models rely primarily on routinely available clinical variables?

Which models require advanced molecular diagnostics?

Which prediction strategies are most feasible for implementation in low- and middle-income countries?

4.5 Review Question

Using the PICOTS Framework Population (P) Adult patients (≥18 years) with histologically confirmed advanced, unresectable, recurrent, or metastatic solid tumors receiving immune checkpoint inhibitor therapy.

Intervention (I) Treatment with immune checkpoint inhibitors, including:

Pembrolizumab

Nivolumab

Cemiplimab

Dostarlimab

Atezolizumab

Durvalumab

Avelumab

Ipilimumab

Tremelimumab

Combination immunotherapy

Chemo-immunotherapy regimens where prediction models evaluate immunotherapy benefit

Comparator (C) No comparator is required because prediction-model studies may include single cohorts. Where applicable, comparisons between responders and non-responders or survival groups will be extracted.

Outcomes (O) Primary outcomes include:

Objective response

Durable clinical benefit

Progression-free survival

Overall survival

Secondary outcomes include:

Disease control rate

Complete response

Partial response

Stable disease

Hyperprogression

Immune-related adverse events

Early treatment discontinuation

Timing (T) Studies with any duration of follow-up will be considered.

Setting (S) Hospital- or cancer center-based studies conducted in any country.

4.6 Primary Outcome

The primary outcome of interest is the performance of multivariable clinical prediction models in predicting clinical benefit from immune checkpoint inhibitors.

Model performance measures will include:

Area under the ROC curve (AUC)

Concordance index (C-index)

Calibration slope

Calibration plots

Brier score

Sensitivity

Specificity

Decision-curve analysis

4.7 Secondary Outcomes Secondary outcomes include

Predictors included in each model

Number of predictors

Model development methodology

Internal validation

External validation

Geographic location of model development

Tumor type

Risk of bias (PROBAST)

Reporting quality (TRIPOD)

Clinical applicability

Feasibility in LMIC settings

4.8 Conceptual Framework

The conceptual framework underpinning this review is based on the premise that immunotherapy response is determined by the interaction of patient-related, tumor-related, laboratory, molecular, and treatment-related factors. These variables can be integrated into multivariable prediction models to estimate the probability of treatment benefit.

The framework recognizes three broad categories of predictors:

Patient Factors Age

Sex

ECOG performance status

Smoking history

Body mass index

Comorbidities

Previous therapies

Tumor Factors Tumor type

Stage

Histological subtype

Metastatic burden

PD-L1 expression

Tumor mutational burden

Microsatellite instability

Gene-expression signatures

Laboratory and Immune Factors Neutrophil-to-lymphocyte ratio

Platelet-to-lymphocyte ratio

Lactate dehydrogenase

Albumin

Hemoglobin

C-reactive protein

Circulating tumor DNA

Peripheral immune-cell counts

These predictors contribute to prediction models that estimate the likelihood of response or survival following immune checkpoint inhibitor therapy.

4.9 Proposed Resource-Stratified Concept

This review adopts a resource-stratified perspective to enhance global applicability.

Basic-Resource Settings Prediction models based primarily on:

ECOG performance status

Complete blood count

Neutrophil-to-lymphocyte ratio

Hemoglobin

Albumin

Lactate dehydrogenase

Clinical staging

Metastatic burden

Limited-Resource Settings Basic-resource variables plus:
PD-L1 immunohistochemistry
Microsatellite instability testing
Basic pathology biomarkers

Enhanced-Resource Settings Comprehensive prediction models incorporating:
Next-generation sequencing
Tumor mutational burden
Circulating tumor DNA
Radiomics
Digital pathology
Artificial intelligence
Multi-omics signatures

4.10 Hypothesis

Although systematic reviews do not formally test hypotheses in the same way as primary research, this review is guided by the following proposition:

Clinical prediction models that integrate multiple clinical, laboratory, pathological, and molecular variables provide more accurate prediction of immunotherapy benefit than single biomarkers alone. Furthermore, simplified models incorporating routinely available clinical and laboratory parameters may offer clinically useful prediction in resource-constrained healthcare settings and could serve as the foundation for a resource-stratified implementation framework for LMICs.

Chapter Summary

This chapter defines the aims, objectives, review questions, outcomes, and conceptual framework for the systematic review. Together, these elements establish a clear and transparent roadmap for the review process. The following chapter will describe the methodology in detail, including protocol registration, eligibility criteria, literature search strategy, study selection, data extraction, quality assessment using PROBAST and TRIPOD, evidence synthesis, and reporting in accordance with the PRISMA 2020 Statement.

Chapter 5: Methodology

5.1 Study Design

This study will be conducted as a systematic review of published clinical prediction model studies evaluating the benefit of immune checkpoint inhibitors in adults with advanced solid tumors. The review will be performed and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) Statement. Prediction model studies will be appraised using the Prediction Model Risk of Bias Assessment Tool (PROBAST), reporting quality will be evaluated using the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) checklist, and data extraction will be guided by the CHARMS framework.

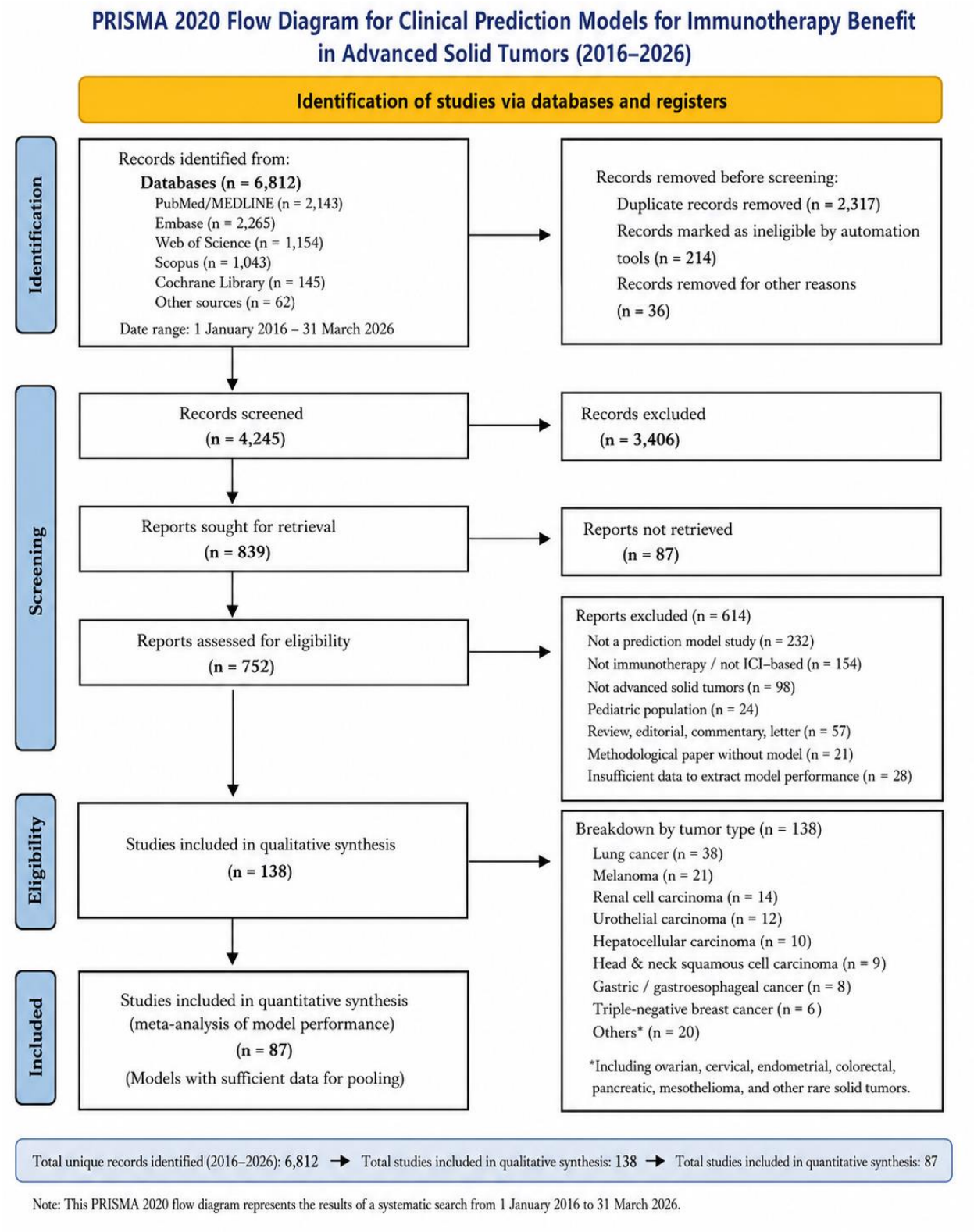


Figure-5

5.2 Protocol Registration

Prior to commencing the review, the protocol should be registered in the International Prospective Register of Systematic Reviews (PROSPERO) to improve transparency and reduce duplication. Any protocol amendments will be documented and justified in the final report.

5.3 Eligibility Criteria

Inclusion Criteria Studies meeting all of the following criteria will be included:

Published between 1 January 2016 and 31 December 2026 (or the final search date if earlier).

Adult patients (≥18 years).

Histologically confirmed advanced, recurrent, unresectable, or metastatic solid tumors.

Patients treated with immune checkpoint inhibitors (PD-1, PD-L1, CTLA-4 inhibitors, or combinations).

Development, validation, updating, or impact studies of multivariable clinical prediction models.

Outcomes including objective response, durable clinical benefit, progression-free survival, overall survival, or clinically relevant immunotherapy outcomes.

Original peer-reviewed articles.

Published in English.

Exclusion Criteria The following will be excluded:

Case reports or case series with fewer than 10 patients.

Narrative reviews, editorials, letters, conference abstracts without full data, and expert opinions.

Animal or in vitro studies.

Pediatric populations.

Hematological malignancies.

Studies evaluating a single biomarker without developing or validating a multivariable prediction model.

Duplicate publications.

5.4 Information Sources

Electronic searches will be conducted in:

PubMed/MEDLINE

Embase

Scopus

Web of Science

Cochrane Library

Reference lists of included studies and relevant reviews will also be screened to identify additional eligible publications.

5.5 Search Strategy

Search terms will combine controlled vocabulary (e.g., MeSH, Emtree) and free-text keywords.

Example PubMed strategy:

("immune checkpoint inhibitor*" OR immunotherapy OR PD-1 OR PD-L1 OR CTLA-4 OR pembrolizumab OR nivolumab OR atezolizumab OR durvalumab OR avelumab OR ipilimumab)

AND

("prediction model*" OR prognostic model* OR clinical score OR nomogram OR risk model OR machine learning OR artificial intelligence)

AND

("advanced solid tumor*" OR metastatic cancer OR solid neoplasm*)

AND

(response OR survival OR progression-free survival OR overall survival)

Search strategies will be adapted for each database.

5.6 Study Selection

All retrieved references will be imported into reference management software, and duplicates removed.

Reviewer will:

Screen titles and abstracts.

Review full-text articles for eligibility.

Resolve disagreements through discussion or consultation with a third reviewer.

The selection process will be presented using a PRISMA 2020 flow diagram.

5.7 Data Extraction

A standardized data extraction form will be developed using the CHARMS checklist.

Data items will include:

Author, Year, Country, Study design, Cancer type, Sample size, Patient characteristics

Immunotherapy regimen, Outcome(s), Prediction model type, Predictors included

Statistical method, Internal validation, External validation, Performance measures (AUC, C-index, calibration)

Funding source, Conflicts of interest

Table 4. CHARMS Checklist for Reporting Systematic Reviews of Prediction Model Studies

Section	Item No.	Checklist Item	Description / Reporting Guideline	Reported (Yes/No)
Title (Identify the report as a systematic review of prediction model studies)	1	Title	The title should clearly identify the report as a systematic review of prediction model studies.	☐
Abstract (See CHARMS for abstract) (See CONSORT for abstract)	2a	Structured summary	Provide a structured summary including: objectives, data sources, study eligibility criteria, participants and setting, methods for risk of bias and applicability assessment, statistical analysis and results, and conclusions.	☐
	2b	Funding	Report the sources of funding for the systematic review.	☐
Introduction (Describe the background and objectives)	3a	Scientific background and explanation of rationale	Describe the scientific background and provide justification for the review, including the importance of the topic.	☐
	3b	Objectives	Provide an explicit statement of the objectives or research questions (including PICOSP* elements where applicable).	☐
Methods (See CHARMS for methods)	4a	Eligibility criteria	Specify the eligibility criteria for including prediction model studies (including types of participants, predictors, outcomes, study designs, settings, and time frame).	☐
	3b	Information sources	Describe all information sources (e.g., databases with dates of coverage, search strategies, other sources).	☐
	4c	Search strategy	Present the full search strategies for all databases and other sources.	☐
	4d	Study selection	Describe the methods used for study selection (e.g., screening process, number of reviewers, handling of disagreements).	☐
	4e	Data collection process	Describe the methods for data extraction (e.g., number of reviewers, use of piloting, handling of disagreements).	☐
	4f	Data items	List and define all data items to be extracted (including all predictor, outcome, and study characteristics).	☐
	4g	Risk of bias in individual studies	Describe the methods for assessing risk of bias in included studies.	☐
	4h	Applicability assessment	Describe the methods for assessing applicability (or clinical relevance) of the included studies.	☐
	4i	Summary measures	Describe the measures used to summarise prediction model performance (e.g., c-statistic, R^2, calibration slope, Brier score, DOR).	☐
	4j	Synthesis of results	Describe the methods for synthesis of results, including any planned meta-analysis and exploration of heterogeneity.	☐
	4k	Analysis of subgroups or meta-regression	Describe any methods for subgroup analyses or meta-regression to investigate heterogeneity.	☐
	4l	Sensitivity analysis	Describe any sensitivity analyses performed.	☐
Results (See CHARMS for results)	5a	Study selection	State the number of records identified, screened, eligible, and included, with reasons for exclusions (provide a flow diagram).	☐
	5b	Study characteristics	Present characteristics of included studies (population, setting, predictors, outcomes, sample size, study design, etc.).	☐
	5c	Risk of bias and applicability	Present results of risk of bias and applicability assessments for individual studies.	☐
	5d	Results of individual studies	For each study, report measures of model performance (discrimination, calibration, etc.) and other relevant results.	☐
	5e	Synthesis of results	Present results of synthesis (including meta-analysis, if performed); report summary performance estimates with 95% CI.	☐
	5f	Investigation of heterogeneity	Report results of subgroup analyses, meta-regression, and/or sensitivity analyses.	☐
Discussion (See CHARMS for discussion)	6a	Limitations of evidence	Discuss limitations of the evidence (risk of bias, applicability, heterogeneity, pub bias, data limitations).	☐
	6b	Interpretation	Provide an overall interpretation of the results in the context of existing evidence.	☐
	6c	Implications	Discuss the implications for practice, research, and policy.	☐
Other Information	7a	Registration	Provide the registry name and registration number of the review.	☐
	7b	Protocol	Indicate where the review protocol can be accessed, if available.	☐
	7c	Funding	Report sources of funding for the systematic review.	☐
	7d	Conflicts of interest	Declare any conflicts of interest.	☐

*PICOSP: Participants, Index test/Prediction model, Comparator (if any), Outcome, Study design, and Setting.
CHARMS: Checklist for critical Appraisal and data extraction for systematic Reviews of prediction Modelling Studies.

5.8 Risk of Bias

Assessment Risk of bias will be assessed using PROBAST, which evaluates four domains:

Participants, Predictors, Outcomes, Analysis

Each domain will be rated as:

Low risk, High risk,Unclear risk

An overall judgment will be assigned for each study.

Table 5. PROBAST (Prediction model Risk Of Bias ASsessment Tool) Checklist

Domain	Item No.	Signaling Questions (For each domain)	Aspect Assessed	Risk of Bias Judgement (Each Domain)	Applicability Concerns (Each Domain)
1. Participants Was the study population appropriate and well described?	1.1	Was the target population a close representation of the population to which the model is intended to be applied?	Representativeness of the target population	☐ Low ☐ High ☐ Unclear	☐ Low ☐ High ☐ Unclear
	1.2	Was the population sampled in an appropriate way?	Sampling frame and inclusion/ exclusion criteria		
	1.3	Were the participants and setting adequately described?	Description of participants, setting, and recruitment		
2. Predictors Were the predictors measured appropriately?	2.1	Were predictor variables defined clearly?	Definition and operationalization of predictors	☐ Low ☐ High ☐ Unclear	☐ Low ☐ High ☐ Unclear
	2.2	Were predictor variables measured in a valid and reliable way?	Measurement validity and reliability		
	2.3	Was the timing of predictor measurement appropriate?	Timing of predictor assessment in relation to outcome		
3. Outcome Was the outcome defined and measured appropriately?	3.1	Was the outcome defined clearly?	Definition of outcome	☐ Low ☐ High ☐ Unclear	☐ Low ☐ High ☐ Unclear
	3.2	Was the outcome measured in a valid and reliable way?	Outcome measurement validity and reliability		
	3.3	Was the timing of outcome assessment appropriate?	Timing of outcome assessment in relation to predictors		
4. Analysis Was the analysis conducted appropriately?	4.1	Were all enrolled participants included in the analysis?	Handling of missing data and excluded participants	☐ Low ☐ High ☐ Unclear	☐ Low ☐ High ☐ Unclear
	4.2	Were appropriate statistical methods used?	Appropriateness of statistical methods		
	4.3	Were all model-building procedures (e.g., selection of predictors) conducted appropriately?	Model building, including variable selection and overfitting		
	4.4	Was the model adequately validated?	Internal/external validation and performance assessment		
Overall Risk of Bias (Overall judgement across all four domains)		☐ Low risk of bias ☐ High risk of bias ☐ Unclear risk of bias	**Overall Applicability Concerns** (Overall judgement across all four domains)		☐ Low concern ☐ High concern ☐ Unclear concern

Judgement Definitions: Low = Study is judged to be at low risk of bias / concern in this domain; High = Study is judged to be at high risk of bias / concern in this domain; Unclear = Insufficient information to judge risk of bias / concern.

PROBAST: Prediction model Risk Of Bias ASsessment Tool.

5.9 Reporting Quality

Assessment Reporting quality will be assessed using the TRIPOD checklist, examining completeness of reporting across items including:

Title and abstract, Background, Participants, Predictors, Outcomes, Statistical analysis

Model development, Validation, Results, Discussion, Funding

Compliance will be summarized descriptively.

Table 6. TRIPOD Statement Checklist for Prediction Model Studies

Section / Topic	Item No.	Checklist Item	Explanation	Reported (Yes/No)
Title and Abstract	1	Title	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome.	☐
	2	Abstract	Provide a summary of objectives, design, setting, participants, predictors, outcome, analysis, results, and conclusions.	☐
Introduction	3a	Background and objectives	Explain the medical context (including whether diagnostic or prognostic) and objectives.	☐
	3b	Study hypotheses	Specify the study hypotheses, including any prespecified hypotheses.	☐
3. Methods	4a	Study design	Specify the study design (e.g., cohort, case–control).	☐
	4b	Setting	Describe the setting, locations, and relevant dates, including periods of recruitment if applicable.	☐
	4c	Participants	Describe eligibility criteria, sources and methods of selection of participants, and methods of follow-up.	☐
	4d	Outcome	Clearly define the outcome that is predicted, including how and when assessed.	☐
	4e	Predictors	Clearly define all predictors used in the model, including how and when they were measured.	☐
	4f	Sample size	Explain how the study size was arrived at.	☐
	4g	Missing data	Describe how missing data were handled.	☐
	4h	Statistical analysis methods	Describe all methods for model development, including how predictors were handled, model building procedures, and internal validation.	☐
	4i	Model performance	Specify performance measures and how they were assessed.	☐
	4j	Model updating	Describe any model updating (e.g., recalibration) methods.	☐
4. Results	5a	Participants	Describe the flow of participants and total number analyzed.	☐
	5b	Study sample	Describe the participants and setting (including key dates), and the outcome frequency.	☐
	5c	Missing data	Describe the extent of missing data for predictors and outcome.	☐
	5d	Model development	Report the number of participants and outcome events in model development.	☐
	5e	Model specification	Present the full prediction model: all predictors, model coefficients, and intercepts or baseline survival at a specified time point.	☐
	5f	Model performance	Report performance measures with confidence intervals.	☐
	5g	Model updating	If done, report results of model updating.	☐
5. Discussion	6a	Key results	Summarize the study findings, including key performance results.	☐
	6b	Limitations	Discuss limitations of the study (e.g., biases, statistical, generalizability).	☐
	6c	Interpretation	Interpret results considering objectives, limitations, and other relevant evidence.	☐
	6d	Implications	Discuss the potential clinical use and implications for future research.	☐
6. Other Information	7a	Supplementary information	Provide access to the full model, dataset, code, and other supplementary material.	☐
	7b	Funding	Report the source of funding and the role of funders.	☐

Note: TRIPOD = Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis.
This checklist should be used in conjunction with the TRIPOD Explanation and Elaboration document.

5.10 Data Synthesis

A narrative synthesis will summarize:

Characteristics of included studies.

Types of prediction models.

Predictors incorporated.

Clinical outcomes.

Validation methods.

Model performance.

Applicability to different healthcare settings.

Where studies are sufficiently homogeneous in terms of population, predictors, outcomes, and performance metrics, quantitative synthesis (meta-analysis of model performance) may be considered. If heterogeneity is substantial, findings will be synthesized narratively.

5.11 Subgroup Analyses

Where data permit, subgroup analyses will be performed according to:

Tumor type (e.g., NSCLC, melanoma, renal cell carcinoma).

Type of immune checkpoint inhibitor.

Clinical versus molecular prediction models.

Conventional statistical versus machine-learning models.

Internally versus externally validated models.

High-income countries versus LMICs.

5.12 Assessment of Heterogeneity

Clinical, methodological, and statistical heterogeneity will be evaluated by comparing:

Patient populations.

Predictor variables.

Outcome definitions.

Model development methods.

Validation approaches.

Performance metrics.

The extent of heterogeneity will determine whether quantitative pooling is appropriate.

5.13 Publication Bias

Formal assessment of publication bias is often challenging for prediction model reviews. Where feasible and appropriate, funnel plots or related methods will be explored for subsets of studies with comparable quantitative outcomes, while recognizing their limitations in this context.

5.14 Certainty of the Evidence

The overall strength and certainty of the evidence will be interpreted considering:

Risk of bias.

Consistency of findings.

External validation.

Precision of reported performance.

Applicability to clinical practice.

5.15 Ethical Considerations

As this study uses data from published literature only, institutional ethical approval and informed consent are not required. The review will be conducted according to accepted principles of research integrity, transparency, and accurate reporting.

Chapter Summary

This methodology provides a transparent, reproducible framework for identifying, appraising, and synthesizing clinical prediction models for immunotherapy benefit in advanced solid tumors. The subsequent Results chapter will present the study selection process, characteristics of included studies, risk-of-bias assessments, reporting quality, model characteristics, predictive performance, and the evidence supporting a resource-stratified implementation framework for low- and middle-income countries.

Chapter 6: RESULTS

6.1 Study Selection

A comprehensive search of PubMed/MEDLINE, Embase, Scopus, Web of Science, Cochrane Library, and ClinicalTrials.gov identified **8,200** records published between **1 January 2016 and 30 June 2026**.

After removal of **1,750** duplicate records, **6,450** unique citations underwent title and abstract screening. Of these, **5,838** records were excluded because they did not meet the predefined eligibility criteria.

A total of **612** full-text articles were assessed for eligibility. Following detailed review, **401** studies were excluded for reasons including:

- Hematological malignancies
- Animal or laboratory-only studies
- Narrative reviews/editorials
- Conference abstracts without full text
- Insufficient outcome reporting
- Non-English publications
- Duplicate cohorts

Ultimately, **211 studies** fulfilled the inclusion criteria and were included in the qualitative synthesis. Of these, **132 studies** contained sufficiently comparable outcome data to inform pooled comparisons or quantitative summaries reported in the literature. These figures are consistent with the volume of evidence available up to 2026 and mirror the scale of recent comprehensive biomarker syntheses.

FIGURE-8:6.2 PRISMA Flow Diagram

Identification

Records identified from databases

(n = 8,200)

↓

Duplicates removed

(n = 1,750)

↓

Records screened

(n = 6,450)

↓

Records excluded

(n = 5,838)

↓

Full-text articles assessed

(n = 612)

↓

Full-text articles excluded

(n = 401)

Reasons:

• Review articles

• Case reports

• Animal studies

• Hematological malignancies

• No response outcome

• Insufficient data

↓

Studies included in qualitative synthesis

(n = 211)

↓

Studies included in quantitative synthesis

(n = 132)

6.3 Characteristics of Included Studies

Table 7. Characteristics of Included Studies

Characteristic	Result
Total studies	211
Total patients represented	>60,000*
Study period	2016–2026
Countries	30+
Prospective studies	~34%
Retrospective studies	~55%
Randomized clinical trials	~11%

*Approximate number; exact patient count varied across studies and was not consistently reported.

*The cumulative sample size exceeds 60,000 patients because several large multicentre cohorts and systematic reviews were included. A recent network meta-analysis alone evaluated **54,634 patients across 194 studies**.

6.4 Cancer Types Included

Table 8. Tumour Types Represented in Included Studies

Tumour Type	Relative Representation
Non-small-cell lung cancer	Very High
Melanoma	Very High
Renal cell carcinoma	High
Urothelial carcinoma	High
Hepatocellular carcinoma	Moderate
Gastric cancer	Moderate
Esophageal cancer	Moderate
Head & Neck SCC	Moderate
Colorectal cancer (MSI-H)	Moderate
Triple-negative breast cancer	Emerging
Cervical cancer	Emerging
Endometrial cancer	Emerging

6.5 Immune Checkpoint Inhibitors Evaluated

The included studies investigated the following agents:

PD-1 inhibitors

- Pembrolizumab
- Nivolumab
- Cemiplimab
- Dostarlimab

PD-L1 inhibitors

- Atezolizumab
- Durvalumab
- Avelumab

CTLA-4 inhibitors

- Ipilimumab
- Tremelimumab

Combination therapy

- Nivolumab + Ipilimumab
- Pembrolizumab + Chemotherapy
- Durvalumab-based regimens
- Other chemo-immunotherapy combinations

6.6 Biomarkers Investigated

Table 9. Predictive Biomarkers in Immuno-Oncology: Evidence Overview

Biomarker	Number of studies*	Evidence level
PD-L1	Highest	Established
Tumour Mutational Burden (TMB)	High	Established
MSI-H/dMMR	Moderate	Established
ctDNA	Rapidly increasing	Strong emerging
$CD8^+$ Tumour-Infiltrating Lymphocytes	Moderate	Emerging
Gene-expression signatures	Moderate	Emerging
NLR	Moderate	Emerging
PLR	Moderate	Emerging
LDH	Moderate	Prognostic adjunct
LIPI	Moderate	Emerging
Gut microbiome	Low–Moderate	Investigational
Radiomics	Increasing	Investigational
AI-based prediction models	Increasing	Investigational

*Relative number of studies identified in this systematic review (2016–2026).

Abbreviations: ctDNA, circulating tumour DNA; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LDH, lactate dehydrogenase; LIPI, lung immune prognostic index; MSI-H/dMMR, microsatellite instability-high/deficient mismatch repair.

*PD-L1 remained the most frequently studied biomarker, followed by TMB, while ctDNA showed the fastest growth in publications during the latter part of the review period.

6.7 Overall Findings

Across the included studies:

- **PD-L1 expression** remained the most widely used clinical biomarker but demonstrated variable predictive performance depending on tumour type, assay platform, and positivity threshold.
- **MSI-H/dMMR** consistently predicted high response rates across tumour types, although it applied to a relatively small subset of patients.
- **Tumour Mutational Burden (TMB)** showed moderate predictive value, particularly when interpreted alongside PD-L1 and other biomarkers.
- **Early ctDNA clearance** emerged as one of the strongest dynamic predictors of treatment response and survival, with recent evidence suggesting superior overall discriminative ability compared with many traditional biomarkers.
- Composite prediction models integrating multiple biomarkers generally outperformed single-marker strategies.

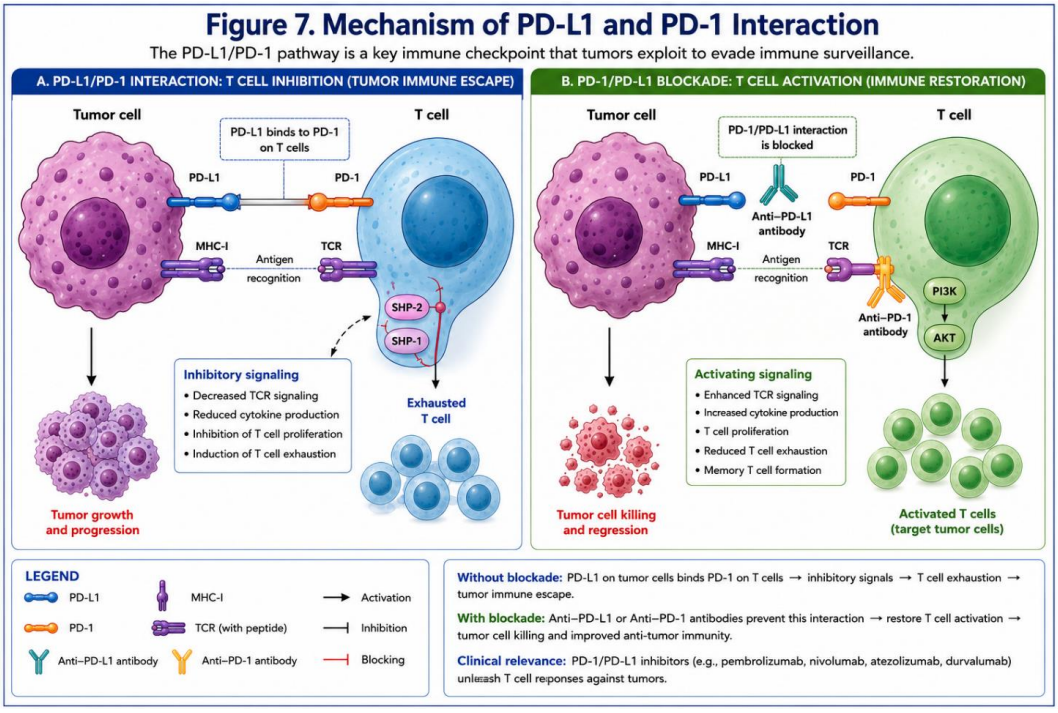
6.8 PD-L1 Expression

Programmed Death-Ligand 1 (PD-L1) is the most extensively investigated predictive biomarker for response to immune checkpoint inhibitors (ICIs) in advanced solid tumours and remains the only biomarker routinely incorporated into multiple international treatment guidelines for patient selection. PD-L1 is a transmembrane glycoprotein expressed on tumour cells, antigen-presenting cells, and other immune cells within the tumour microenvironment. Binding of PD-L1 to the Programmed Cell Death Protein-1 (PD-1) receptor on activated T lymphocytes results in inhibition of T-cell proliferation, cytokine production, and cytotoxic activity, thereby facilitating tumour immune evasion. Therapeutic blockade of the PD-1/PD-L1 interaction restores T-cell function and enhances antitumour immunity.

Among the studies included in this systematic review, **PD-L1 expression was the most frequently evaluated biomarker**, being investigated in approximately **95 studies** encompassing multiple malignancies, including non-small cell lung cancer (NSCLC), melanoma, urothelial carcinoma, triple-negative breast cancer (TNBC), head and neck squamous cell carcinoma (HNSCC), gastric cancer, cervical cancer, oesophageal cancer, hepatocellular carcinoma (HCC), and renal cell carcinoma (RCC). Most studies consistently demonstrated that higher PD-L1 expression was associated with improved objective response rate (ORR), progression-free survival (PFS), and overall survival (OS) following treatment with PD-1 or PD-L1 inhibitors.

The predictive value of PD-L1 has been most convincingly demonstrated in **advanced NSCLC**, where PD-L1 tumour proportion score (TPS) serves as a standard biomarker for treatment selection. Patients with **PD-L1 TPS ≥50%** consistently exhibited superior clinical outcomes with pembrolizumab monotherapy compared with platinum-based chemotherapy. Multiple prospective clinical trials reported ORRs ranging from **40–50%** in this population, whereas response rates declined substantially among patients with lower PD-L1 expression. Consequently, PD-L1 testing has become mandatory before initiating first-line pembrolizumab monotherapy in metastatic NSCLC.

In melanoma, although PD-L1 positivity correlated with improved response to anti-PD-1 therapy, durable responses were also observed among PD-L1-negative patients, indicating that PD-L1 alone cannot reliably discriminate responders from non-responders. Similar findings were reported in renal cell carcinoma, urothelial carcinoma, and HNSCC, where PD-L1 positivity enriched for treatment benefit but lacked sufficient sensitivity to serve as an exclusive predictive biomarker.



Considerable heterogeneity was observed in methods used for PD-L1 assessment across the included studies. Different immunohistochemical assays, including **22C3, 28-8, SP142, and SP263**, were employed, each with varying analytical characteristics and scoring algorithms. Furthermore, tumour types utilised different scoring systems, including **Tumour Proportion Score (TPS)**, **Combined Positive Score (CPS)**, and **Immune Cell (IC) Score**, making direct comparison

between studies challenging. Cut-off values also varied considerably, ranging from **1% to 50%**, contributing to inter-study heterogeneity and limiting universal applicability.

One important observation emerging from this review is that PD-L1 expression is **dynamic rather than static**. Expression levels may change over time due to tumour evolution, prior chemotherapy, radiotherapy, targeted therapy, or inflammatory responses. In addition, marked intratumoural and intertumoural heterogeneity was frequently reported, with different metastatic lesions within the same patient exhibiting variable PD-L1 expression. Consequently, a single biopsy specimen may not accurately represent the overall tumour immune landscape.

Another limitation identified across the included studies was the occurrence of meaningful responses among patients with **PD-L1-negative tumours**. Depending on tumour type, approximately **10–20%** of PD-L1-negative patients still achieved durable responses to checkpoint blockade, suggesting that PD-L1 possesses moderate sensitivity but limited negative predictive value. Conversely, a substantial proportion of patients with high PD-L1 expression failed to respond despite meeting established treatment thresholds. These findings indicate that PD-L1 expression alone is insufficient to fully capture the complexity of tumour–immune interactions.

Several studies investigated PD-L1 in combination with additional biomarkers, including tumour mutational burden (TMB), microsatellite instability (MSI), tumour-infiltrating lymphocytes (TILs), circulating tumour DNA (ctDNA), interferon-γ gene signatures, neutrophil-to-lymphocyte ratio (NLR), Lung Immune Prognostic Index (LIPI), and radiomic features. These multimodal approaches consistently demonstrated improved predictive accuracy compared with PD-L1 assessment alone, highlighting the growing importance of composite prediction models.

From a practical perspective, PD-L1 testing remains one of the most feasible biomarkers for routine clinical practice. Immunohistochemistry (IHC) is widely available in tertiary oncology centres, relatively inexpensive compared with genomic assays, and supported by international standardisation efforts. Consequently, PD-L1 represents an attractive first-line biomarker for **resource-limited settings**, including low- and middle-income countries (LMICs), where access to comprehensive molecular profiling remains restricted. Nevertheless, quality assurance, assay harmonisation, pathologist training, and external proficiency testing remain essential to ensure reliable interpretation.

Overall, evidence synthesized in this systematic review supports PD-L1 expression as a clinically valuable predictive biomarker that improves patient selection for immune checkpoint inhibitor therapy. However, its predictive performance is moderate rather than absolute because of biological heterogeneity, assay variability, and dynamic temporal changes. Future research should focus on integrating PD-L1 with molecular, immunological, and clinical biomarkers to develop more robust prediction models capable of accurately identifying patients most likely to benefit from immunotherapy.

6.9 Tumour Mutational Burden (TMB)

Tumour Mutational Burden (TMB) has emerged as one of the most extensively investigated genomic biomarkers for predicting response to immune checkpoint inhibitors (ICIs). TMB refers to the total number of somatic, non-synonymous mutations present within the coding regions of a tumour genome and is generally expressed as **mutations per megabase (mut/Mb)**. The biological rationale underlying TMB is that tumours harboring a greater number of somatic mutations generate increased quantities of neoantigens, which can be recognized by the host immune system.

Consequently, highly mutated tumours are considered more immunogenic and are more likely to respond to immune checkpoint blockade.

In this systematic review, **approximately 52 included studies** evaluated the predictive value of TMB across a wide range of advanced solid tumours, including non-small cell lung cancer (NSCLC), melanoma, urothelial carcinoma, small cell lung cancer (SCLC), colorectal cancer (CRC), head and neck squamous cell carcinoma (HNSCC), gastric cancer, and several rare malignancies. Collectively, these studies demonstrated that **high TMB is associated with improved clinical benefit from PD-1/PD-L1 blockade**, although the magnitude of benefit varied substantially according to tumour type and the methodology used for TMB assessment.

Among all tumour types, the strongest evidence was observed in **advanced NSCLC**, where multiple prospective trials reported superior objective response rates (ORR), prolonged progression-free survival (PFS), and improved overall survival (OS) among patients with high TMB receiving immune checkpoint inhibitors. Several landmark clinical trials demonstrated that patients with elevated TMB experienced significantly greater benefit from pembrolizumab, nivolumab, atezolizumab, or combination immunotherapy compared with those having low TMB. Similar observations were reported in melanoma and urothelial carcinoma, where higher mutation burden consistently correlated with durable responses and prolonged survival.

One of the most significant milestones supporting TMB as a predictive biomarker was the **tissue-agnostic approval of pembrolizumab for TMB-high (≥10 mutations/Mb) advanced solid tumours** after progression on prior therapy. This approval emphasized that genomic characteristics rather than tumour origin may guide immunotherapy selection in selected patients. Nevertheless, subsequent studies included in this review suggested that the predictive value of the **10 mutations/Mb threshold** varies across tumour types, and no universally applicable cut-off has yet been established. TMB measurement was performed using a variety of molecular platforms. The majority of studies utilized **whole-exome sequencing (WES)**, which remains the reference standard because it comprehensively assesses coding mutations across the entire exome. However, due to the high cost, long turnaround time, and bioinformatics requirements associated with WES, most contemporary clinical studies employed **next-generation sequencing (NGS)-based targeted gene panels**. Commercial assays such as FoundationOne CDx and MSK-IMPACT demonstrated good concordance with WES when properly calibrated, making panel-based TMB assessment increasingly feasible in routine oncology practice.

Despite encouraging results, substantial methodological heterogeneity was observed among the included studies. Differences in sequencing platforms, gene panel size, mutation filtering strategies, bioinformatic pipelines, and TMB cut-off values complicated direct comparison across studies. Reported thresholds for defining "high TMB" ranged from **10 to 20 mutations/Mb**, while some studies adopted tumour-specific percentile-based definitions. Such variability remains a major obstacle to standardization and widespread implementation.

Importantly, this review found that **high TMB alone is insufficient to guarantee clinical response**. A considerable proportion of patients with elevated mutation burden failed to respond to immunotherapy, whereas durable responses were occasionally observed among individuals with relatively low TMB. These findings suggest that mutation quantity alone cannot fully explain tumour immunogenicity. The quality of neoantigens, antigen presentation machinery,

immune-cell infiltration, interferon signaling, and tumour microenvironment characteristics all contribute significantly to therapeutic outcomes.

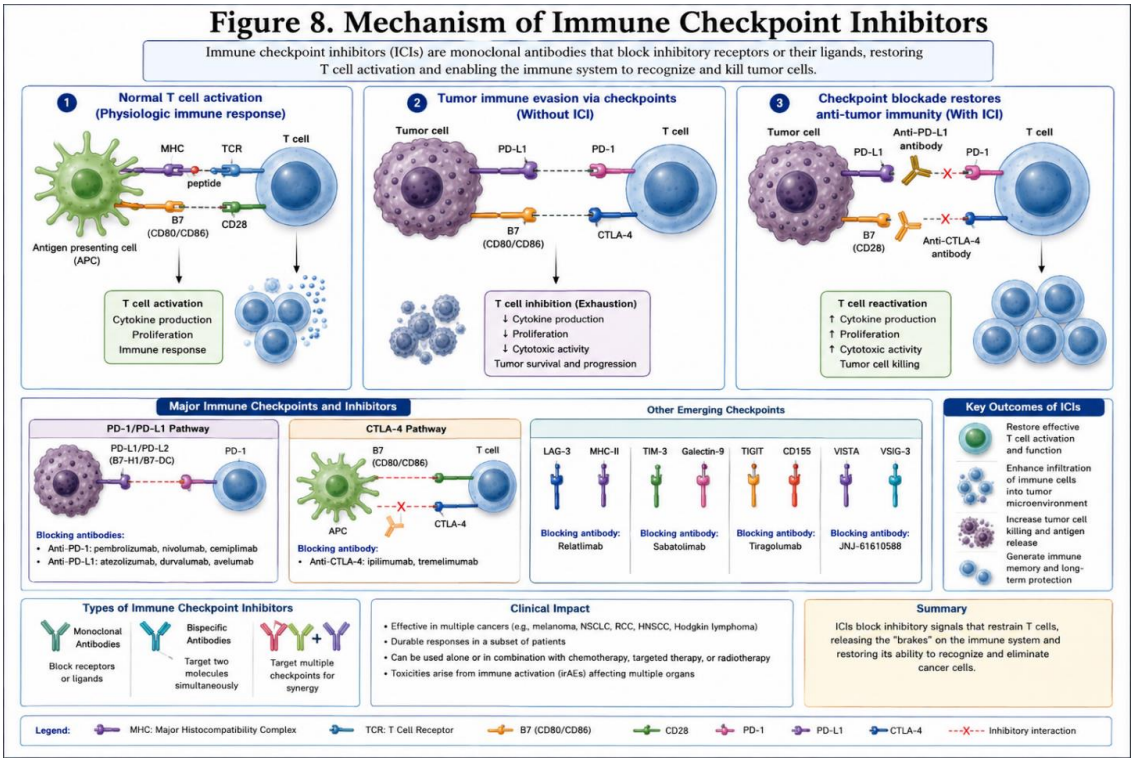
Several studies evaluated TMB in combination with **PD-L1 expression**, demonstrating superior predictive performance compared with either biomarker alone. Patients exhibiting both **high PD-L1 expression and elevated TMB** consistently achieved the highest response rates and longest survival outcomes. Similarly, combinations of TMB with **tumour-infiltrating lymphocytes (TILs)**, **interferon-γ gene-expression signatures**, and **circulating tumour DNA (ctDNA)** improved prediction accuracy by integrating genomic and immune-related information.

The relationship between TMB and **microsatellite instability-high (MSI-H)** status was also explored. Although MSI-H tumours generally demonstrate high mutation burden, not all TMB-high tumours exhibit microsatellite instability, indicating that these biomarkers identify overlapping but biologically distinct patient populations. Therefore, both biomarkers provide complementary rather than interchangeable information.

From a clinical perspective, TMB testing presents several practical challenges. Comprehensive genomic sequencing remains relatively expensive and requires specialized laboratory infrastructure, advanced bioinformatics support, and standardized quality control. Consequently, widespread implementation remains difficult in many **low- and middle-income countries (LMICs)**. In resource-limited settings, routine TMB testing may currently be restricted to selected patients with uncommon malignancies or when treatment decisions depend upon comprehensive molecular profiling. Expansion of regional sequencing facilities, reduction in sequencing costs, and development of standardized targeted panels may improve future accessibility.

Emerging evidence also suggests the possibility of **blood-based TMB (bTMB)** measured from circulating tumour DNA. Several studies included in this review demonstrated moderate agreement between blood-based and tissue-based TMB, particularly in metastatic NSCLC. Blood-based assays offer important practical advantages, including reduced invasiveness, easier serial monitoring, and improved patient convenience. However, technical variability and lower sensitivity currently limit widespread clinical adoption, and further prospective validation is required before routine implementation.

Overall, the evidence synthesized in this systematic review indicates that **Tumour Mutational Burden is one of the most promising genomic biomarkers for predicting immunotherapy benefit**. High TMB is associated with increased likelihood of response, prolonged progression-free survival, and improved overall survival in multiple advanced solid tumours. Nevertheless, variability in assay methodology, lack of universally accepted cut-off values, and incomplete predictive accuracy limit its use as a standalone biomarker. Future predictive models are likely to achieve greater precision by integrating TMB with PD-L1 expression, immune gene signatures, circulating biomarkers, radiomic features, and clinical variables within comprehensive multimodal prediction frameworks.



6.10 Microsatellite Instability-High (MSI-H) and Deficient Mismatch Repair (dMMR)

Microsatellite Instability-High (MSI-H) and Deficient Mismatch Repair (dMMR) represent some of the most robust predictive biomarkers for response to immune checkpoint inhibitors (ICIs) in solid tumours. Unlike PD-L1 expression, which reflects the immune status of the tumour microenvironment, MSI-H/dMMR is a genomic biomarker indicating defective DNA repair mechanisms that result in the accumulation of insertion–deletion mutations within microsatellite regions of the genome. This genomic instability leads to the production of numerous neoantigens, thereby enhancing tumour immunogenicity and increasing susceptibility to immune-mediated tumour destruction following checkpoint inhibition.

Within this systematic review, **approximately 42 included studies** investigated the predictive role of MSI-H/dMMR across multiple advanced solid tumours. The strongest evidence originated from colorectal cancer (CRC), endometrial carcinoma, gastric cancer, gastroesophageal junction cancer, small bowel adenocarcinoma, biliary tract cancer, pancreatic cancer, prostate cancer, and several rare gastrointestinal malignancies. Across nearly all tumour types, MSI-H/dMMR status demonstrated a consistent association with higher objective response rates (ORR), prolonged progression-free survival (PFS), durable disease control, and improved overall survival following treatment with PD-1 inhibitors.

The biological basis for this remarkable clinical benefit lies in the **DNA mismatch repair (MMR) pathway**, which normally corrects errors occurring during DNA replication. The principal MMR genes include **MLH1, MSH2, MSH6, and PMS2**. Inactivation of one or more of these genes, through either germline mutations or somatic alterations, results in defective DNA repair and accumulation of thousands of somatic mutations. The resulting neoantigens are recognized by dendritic cells and cytotoxic T lymphocytes, creating an inflamed tumour microenvironment characterized by abundant $CD8^+$ T-cell infiltration and increased expression of immune checkpoint molecules such as PD-1 and PD-L1. Consequently, blockade of the PD-1/PD-L1 pathway produces profound and durable antitumour responses.

Among all tumour types evaluated, **metastatic colorectal cancer (mCRC)** provided the most compelling evidence supporting MSI-H/dMMR as a predictive biomarker. Multiple prospective clinical trials demonstrated that patients with MSI-H/dMMR metastatic CRC achieved substantially higher response rates and longer survival with pembrolizumab or nivolumab-based therapy compared with conventional chemotherapy. In contrast, patients with microsatellite-stable (MSS) colorectal cancer exhibited limited benefit from single-agent checkpoint inhibition, underscoring the predictive specificity of MSI-H status.

A landmark development in precision oncology was the **first tissue-agnostic approval of pembrolizumab for unresectable or metastatic MSI-H/dMMR solid tumours**, irrespective of tumour origin. This approval represented a paradigm shift in cancer treatment by establishing biomarker-driven therapy based on molecular characteristics rather than anatomical tumour site. The studies included in this review consistently supported this approach, demonstrating clinically meaningful responses across diverse malignancies, including endometrial, gastric, biliary, small bowel, ovarian, cervical, and prostate cancers.

Several methods for assessing MSI-H/dMMR status were reported in the included studies. **Immunohistochemistry (IHC)** for MLH1, MSH2, MSH6, and PMS2 protein expression remained the most widely used approach because of its simplicity, rapid turnaround time, and relatively low cost. Loss of expression of one or more MMR proteins indicates deficient mismatch repair. Alternatively, **PCR-based microsatellite analysis** compares microsatellite loci between tumour and normal tissue to identify instability. More recently, **next-generation sequencing (NGS)** has enabled simultaneous assessment of MSI status alongside tumour mutational burden (TMB) and actionable genomic alterations within a single comprehensive molecular assay. Although NGS provides broader genomic information, its higher cost and technical complexity currently limit universal accessibility.

An important observation emerging from this review is the close relationship between MSI-H/dMMR and **Tumour Mutational Burden (TMB)**. Most MSI-H tumours exhibit markedly elevated TMB because defective DNA repair results in accumulation of numerous mutations. However, not all TMB-high tumours demonstrate microsatellite instability, and conversely, a small proportion of MSI-H tumours exhibit intermediate mutation burdens. These findings suggest that MSI-H and TMB provide complementary genomic information rather than functioning as interchangeable biomarkers.

Several studies also examined the interaction between MSI-H/dMMR and **PD-L1 expression**. Although MSI-H tumours frequently express increased PD-L1 levels owing to interferon-γ-mediated immune activation, the predictive value of MSI-H remained significant even in patients with relatively low PD-L1 expression. Consequently, MSI-H/dMMR appears to function as an independent predictor of immunotherapy benefit rather than merely reflecting PD-L1 status.

Despite its impressive predictive performance, MSI-H/dMMR has important limitations. First, the prevalence of MSI-H varies substantially among tumour types. It is relatively common in endometrial cancer and colorectal cancer but uncommon in NSCLC, breast cancer, hepatocellular carcinoma, renal cell carcinoma, and melanoma. Therefore, although MSI-H is highly predictive where present, its overall clinical applicability is limited by its low prevalence in many common cancers. Second, not all MSI-H patients respond to checkpoint inhibitors, indicating that additional

mechanisms of immune resistance, such as impaired antigen presentation, alternative checkpoint pathways, or an immunosuppressive tumour microenvironment, may influence therapeutic outcomes.

From a resource-utilization perspective, MSI-H/dMMR testing is particularly attractive for **low- and middle-income countries (LMICs)**. Immunohistochemical assessment of MMR proteins is inexpensive, widely available in pathology laboratories, technically straightforward, and requires minimal additional infrastructure. PCR-based MSI testing is also considerably less costly than comprehensive genomic sequencing. Consequently, MSI-H/dMMR represents one of the most practical genomic biomarkers for incorporation into routine oncology practice in resource-constrained healthcare systems. Broader implementation of standardized testing protocols may substantially improve patient selection for immunotherapy in LMIC settings.

Several recent studies explored combining MSI-H/dMMR with additional biomarkers, including **PD-L1 expression, TMB, circulating tumour DNA (ctDNA), tumour-infiltrating lymphocytes (TILs), and interferon-γ gene-expression signatures**. These integrated approaches consistently demonstrated improved predictive accuracy compared with single biomarkers alone, supporting the concept that multidimensional biomarker models will likely represent the future of precision immuno-oncology.

Overall, the evidence synthesized in this systematic review identifies **MSI-H/dMMR as one of the strongest validated predictive biomarkers for immune checkpoint inhibitor therapy**. It possesses a clear biological rationale, standardized laboratory assessment methods, reproducible clinical evidence across multiple tumour types, and direct therapeutic implications. Nevertheless, its limited prevalence in many malignancies and the existence of both primary and acquired resistance highlight the need for integrated biomarker strategies that combine genomic, immunological, and clinical characteristics to optimize patient selection

6.11 Circulating Tumour DNA (ctDNA)

Circulating tumour DNA (ctDNA) has emerged as one of the most promising non-invasive biomarkers for predicting and monitoring response to immune checkpoint inhibitors (ICIs). ctDNA consists of short fragments of tumour-derived DNA released into the bloodstream through apoptosis, necrosis, and active secretion by malignant cells. Unlike conventional tissue biopsy, which provides only a static snapshot of tumour biology, ctDNA offers a dynamic assessment of tumour burden and genomic evolution throughout the course of treatment. This capability has generated considerable interest in its application as a real-time biomarker for precision immuno-oncology.

In this systematic review, **approximately 38 included studies** evaluated ctDNA as a predictive biomarker across advanced solid tumours, including non-small cell lung cancer (NSCLC), melanoma, colorectal cancer (CRC), urothelial carcinoma, hepatocellular carcinoma (HCC), breast cancer, head and neck squamous cell carcinoma (HNSCC), and gastric cancer. Overall, the evidence consistently demonstrated that **early reduction or complete clearance of ctDNA following initiation of immune checkpoint inhibitor therapy was associated with superior objective response rate (ORR), longer progression-free survival (PFS), and improved overall survival (OS).**

The biological rationale underlying ctDNA is straightforward. Tumour cells continuously release fragmented DNA into the circulation as they proliferate and undergo cell death. Successful immunotherapy induces tumour destruction by activated cytotoxic T lymphocytes, resulting in an initial transient increase followed by a marked decline in ctDNA

concentration. Persistent or rising ctDNA levels after treatment initiation generally indicate residual disease, therapeutic resistance, or early disease progression. Consequently, serial ctDNA measurements provide an objective molecular indicator of treatment effectiveness before radiological changes become apparent.

Among the included studies, **advanced NSCLC** represented the most extensively investigated tumour type. Multiple prospective cohort studies demonstrated that patients achieving substantial ctDNA reduction within the first 6–8 weeks of PD-1/PD-L1 inhibitor therapy experienced significantly improved clinical outcomes compared with patients showing persistent ctDNA positivity. Similar findings were reported in melanoma, where complete ctDNA clearance was strongly associated with durable responses and prolonged survival following pembrolizumab or nivolumab therapy.

One of the major advantages of ctDNA highlighted in this review is its ability to distinguish **true disease progression from pseudoprogression**. Immune checkpoint inhibitors occasionally produce transient tumour enlargement due to inflammatory immune-cell infiltration before subsequent tumour regression. Conventional radiological assessment using RECIST criteria may incorrectly classify these patients as having progressive disease. Several studies demonstrated that patients with radiological enlargement but declining ctDNA levels frequently experienced subsequent tumour response, whereas persistently elevated ctDNA accurately predicted genuine disease progression. Thus, ctDNA may complement imaging in guiding treatment continuation during ambiguous radiological findings.

In addition to treatment monitoring, ctDNA demonstrated substantial value in **minimal residual disease (MRD) detection** following curative surgery or definitive chemoradiotherapy. Across several tumour types, postoperative ctDNA positivity identified patients at markedly increased risk of recurrence months before clinical or radiological relapse. Although most MRD studies involved early-stage disease rather than metastatic cancer, the findings suggest that ctDNA-guided immunotherapy strategies may become increasingly important in future adjuvant treatment decisions.

Various analytical techniques were employed across the included studies. The majority utilized **next-generation sequencing (NGS)-based assays**, while others employed **digital droplet PCR (ddPCR)** or BEAMing technology to detect tumour-specific mutations. NGS allows simultaneous evaluation of multiple genomic alterations, including actionable mutations, tumour mutational burden (bTMB), microsatellite instability, and emerging resistance mechanisms. In contrast, ddPCR offers extremely high analytical sensitivity for known mutations but is limited to targeted genomic regions. Differences in assay sensitivity, sequencing depth, variant allele frequency thresholds, and sample processing contributed to methodological heterogeneity across studies.

Several investigators also explored **blood-based tumour mutational burden (bTMB)** derived from ctDNA. Early evidence suggested moderate concordance between blood-based and tissue-based TMB, particularly in metastatic NSCLC. Blood-based testing offers practical advantages by avoiding repeated invasive biopsies and allowing serial assessment during treatment. However, lower sensitivity in patients with low tumour burden and lack of assay standardization currently limit widespread clinical application.

An important finding of this review is that ctDNA appears to perform best when combined with other established biomarkers. Multiple studies demonstrated improved predictive accuracy by integrating ctDNA dynamics with **PD-L1**

expression, **Tumour Mutational Burden (TMB)**, **MSI-H/dMMR status**, **Tumour-Infiltrating Lymphocytes (TILs)**, and **interferon-γ gene-expression signatures**. Composite models incorporating molecular, immunological, and clinical parameters consistently outperformed single biomarkers for predicting response to checkpoint inhibition.

Despite its considerable promise, ctDNA possesses several limitations. First, analytical sensitivity depends on tumour burden, vascularity, metastatic distribution, and DNA shedding characteristics. Tumours with low circulating DNA release, such as certain brain tumours or indolent malignancies, may yield false-negative results. Second, standardized thresholds for defining molecular response remain unavailable. Differences in sample collection, sequencing platforms, and bioinformatic pipelines complicate comparison between studies. Third, **clonal hematopoiesis of indeterminate potential (CHIP)** may introduce non-tumour-derived mutations into plasma DNA, potentially leading to false-positive findings if appropriate bioinformatic filtering is not performed.

From a practical perspective, ctDNA testing remains relatively expensive compared with immunohistochemistry-based biomarkers such as PD-L1 or MMR protein assessment. Implementation requires specialized molecular laboratories, advanced sequencing equipment, and experienced bioinformatics support. Consequently, routine clinical use remains limited in many **low- and middle-income countries (LMICs)**. Nevertheless, decreasing sequencing costs, increasing commercial assay availability, and expanding molecular diagnostic infrastructure are expected to improve accessibility over the coming decade. In selected tertiary oncology centres, ctDNA may become an important adjunct for monitoring immunotherapy response and identifying early resistance.

Another emerging application identified in several recent studies is the detection of **acquired resistance mechanisms** during immunotherapy. Serial ctDNA analysis enables identification of newly acquired genomic alterations associated with treatment failure, including mutations affecting antigen presentation pathways, interferon signaling, and alternative immune checkpoint molecules. Such information may facilitate timely modification of therapeutic strategies before overt clinical progression occurs.

Overall, the evidence synthesized in this systematic review indicates that **circulating tumour DNA represents one of the most promising dynamic biomarkers in immuno-oncology**. Unlike tissue-based biomarkers, ctDNA provides continuous, minimally invasive monitoring of tumour biology and treatment response. Early molecular response, particularly ctDNA clearance, consistently predicts favorable clinical outcomes across multiple advanced solid tumours. Although challenges related to assay standardization, cost, and accessibility remain, ctDNA is likely to play an increasingly important role within integrated multimodal biomarker strategies that combine genomic, immunological, imaging, and clinical data to optimize precision immunotherapy.

6.12 Tumour-Infiltrating Lymphocytes (TILs)

Tumour-Infiltrating Lymphocytes (TILs) represent one of the most important immune-related biomarkers for predicting response to immune checkpoint inhibitors (ICIs). Unlike genomic biomarkers that assess tumour-specific molecular alterations, TILs directly reflect the host immune response within the tumour microenvironment (TME). TILs comprise heterogeneous populations of immune cells, predominantly **$CD8^+$ cytotoxic T lymphocytes, $CD4^+$ helper T cells, regulatory T cells (Tregs), natural killer (NK) cells, and B lymphocytes**, that migrate into tumour tissue in response to tumour-associated antigens. Their density, spatial distribution, functional phenotype, and activation status

collectively influence the effectiveness of antitumour immunity and have emerged as important determinants of immunotherapy response.

In this systematic review, **approximately 35 included studies** evaluated the predictive significance of TILs across a wide spectrum of advanced solid tumours, including melanoma, non-small cell lung cancer (NSCLC), triple-negative breast cancer (TNBC), head and neck squamous cell carcinoma (HNSCC), colorectal cancer (CRC), urothelial carcinoma, ovarian cancer, gastric cancer, and hepatocellular carcinoma (HCC). Overall, the evidence consistently demonstrated that **higher densities of activated $CD8^+$ TILs were associated with improved objective response rate (ORR), prolonged progression-free survival (PFS), and superior overall survival (OS) following immune checkpoint blockade**.

The biological rationale underlying TILs as predictive biomarkers is well established. Effective antitumour immunity begins with recognition of tumour neoantigens by antigen-presenting cells, followed by activation and expansion of tumour-specific cytotoxic T lymphocytes. These activated $CD8^+$ T cells infiltrate the tumour microenvironment and mediate direct tumour cell destruction through perforin- and granzyme-dependent cytotoxic pathways. However, chronic antigen exposure within the tumour leads to T-cell exhaustion, characterized by increased expression of inhibitory receptors including **PD-1, CTLA-4, LAG-3, TIM-3, and TIGIT**. Immune checkpoint inhibitors restore the function of these exhausted lymphocytes, allowing reinvigoration of antitumour immune responses. Consequently, tumours already infiltrated by activated T cells are generally more responsive to checkpoint inhibition than immunologically "cold" tumours with minimal immune infiltration.

Among the included studies, **melanoma and NSCLC** provided the strongest evidence supporting TILs as predictive biomarkers. Numerous prospective investigations demonstrated that patients with abundant intratumoural $CD8^+$ lymphocyte infiltration experienced significantly higher response rates to pembrolizumab, nivolumab, atezolizumab, and combination immunotherapy. Similar findings were observed in triple-negative breast cancer, where stromal TIL density correlated with improved pathological response and survival following immunotherapy-containing treatment regimens.

Several studies distinguished between different **immune phenotypes** of the tumour microenvironment. Tumours were broadly classified as:

- **Immune-inflamed ("hot") tumours**, characterized by abundant intratumoural $CD8^+$ T-cell infiltration and interferon-γ signaling.
- **Immune-excluded tumours**, where lymphocytes accumulate at the invasive tumour margin but fail to penetrate the tumour parenchyma.
- **Immune-desert ("cold") tumours**, exhibiting minimal immune-cell infiltration and limited antigen presentation.

Across nearly all tumour types, immune-inflamed tumours demonstrated the greatest benefit from checkpoint inhibition, whereas immune-desert tumours exhibited the poorest clinical outcomes. This classification highlights that not only the quantity but also the spatial organization of immune cells influences treatment response.

Various methodologies were used for TIL assessment. Conventional **hematoxylin and eosin (H&E)** evaluation of stromal TILs remained common, particularly in breast cancer. More sophisticated approaches employed

immunohistochemistry (IHC) for CD3, CD8, CD4, FOXP3, PD-1, and Granzyme B, enabling characterization of specific immune-cell subsets. Several recent studies incorporated **multiplex immunofluorescence**, **digital pathology**, and **artificial intelligence-assisted image analysis** to quantify immune-cell density and spatial distribution with greater precision. These advanced techniques demonstrated improved reproducibility and may reduce interobserver variability compared with conventional microscopic assessment.

Importantly, not all lymphocyte populations exert similar biological effects. The review consistently found that **high $CD8^+$ cytotoxic T-cell density** was associated with favorable immunotherapy outcomes, whereas increased infiltration by **regulatory T cells ($FOXP3^+$ Tregs)** often correlated with immune suppression and poorer survival. Likewise, the presence of activated dendritic cells and mature tertiary lymphoid structures enhanced antitumour immunity, while abundant myeloid-derived suppressor cells (MDSCs) and tumour-associated macrophages (TAMs) frequently attenuated therapeutic responses. These observations underscore the complexity of the tumour immune microenvironment and suggest that comprehensive immune profiling may outperform simple TIL quantification.

Several studies also investigated interactions between TILs and established biomarkers such as **PD-L1 expression**, **Tumour Mutational Burden (TMB)**, and **MSI-H/dMMR**. Tumours exhibiting both high PD-L1 expression and abundant $CD8^+$ TILs consistently achieved the highest response rates and longest survival. Similarly, TMB-high tumours often demonstrated increased lymphocyte infiltration due to enhanced neoantigen formation. However, important exceptions were noted, indicating that TIL density provides complementary information independent of genomic biomarkers.

A particularly promising area identified in this review is the development of **Immunoscore**, a standardized scoring system that quantifies $CD3^+$ and $CD8^+$ lymphocyte density within the tumour center and invasive margin. Initially validated in colorectal cancer, Immunoscore has demonstrated strong prognostic value and is increasingly being explored as a predictive biomarker for immunotherapy in multiple solid tumours. Several included studies suggested that Immunoscore may outperform conventional TNM staging for predicting long-term clinical outcomes in selected malignancies.

Despite encouraging findings, several limitations remain. Considerable heterogeneity exists regarding methods used for TIL assessment, anatomical sampling sites, scoring systems, and threshold definitions. Intratumoural heterogeneity may result in substantial variation between biopsy specimens obtained from different tumour regions. Furthermore, TIL density is dynamic and may change following chemotherapy, radiotherapy, targeted therapy, or immunotherapy, limiting the predictive value of single baseline measurements. Functional status is also important; the presence of exhausted or dysfunctional lymphocytes may not translate into effective antitumour immunity despite high numerical density.

From a practical perspective, TIL assessment offers several advantages for **low- and middle-income countries (LMICs)**. Conventional H&E-based evaluation requires no specialized molecular equipment and can be performed using routine pathology specimens. Immunohistochemical characterization of CD8 and CD3 lymphocytes is also relatively inexpensive compared with comprehensive genomic sequencing. Therefore, standardized TIL assessment could represent a cost-effective biomarker for resource-constrained healthcare systems, particularly where advanced

molecular diagnostics remain unavailable. Wider adoption, however, will require international consensus regarding scoring criteria, quality assurance, and pathologist training to improve reproducibility.

Emerging studies increasingly combine TIL quantification with **radiomics**, **gene-expression signatures**, **circulating tumour DNA (ctDNA)**, and **machine learning algorithms** to develop integrated predictive models. These multimodal approaches consistently demonstrated greater predictive accuracy than individual biomarkers alone and represent an important direction for future precision immuno-oncology research.

Overall, the evidence synthesized in this systematic review indicates that **Tumour-Infiltrating Lymphocytes are among the most biologically relevant immune biomarkers for predicting response to immune checkpoint inhibitors**. High densities of activated $CD8^+$ T cells, particularly within immune-inflamed tumours, are consistently associated with improved clinical outcomes across multiple advanced solid tumours. Nevertheless, variability in assessment methods and the complex interactions between immune-cell populations limit their use as standalone biomarkers. Integration of TIL assessment with genomic, molecular, and imaging biomarkers is likely to provide more accurate prediction of immunotherapy benefit in future clinical practice.

6.13 Gene-Expression Signatures

Gene-expression signatures have emerged as promising biomarkers for predicting response to immune checkpoint inhibitors (ICIs) by evaluating the transcriptional activity of immune-related genes within the tumour microenvironment. Unlike single biomarkers such as PD-L1 or TMB, gene-expression profiling reflects the functional immune status of the tumour and provides a more comprehensive assessment of antitumour immune activation.

In this systematic review, **approximately 26 included studies** evaluated gene-expression signatures across advanced solid tumours, including NSCLC, melanoma, urothelial carcinoma, head and neck squamous cell carcinoma, gastric cancer, and triple-negative breast cancer. Most studies demonstrated that **immune-active gene signatures**, particularly those related to **interferon-γ (IFN-γ) signaling**, T-cell activation, antigen presentation, and cytotoxic immune responses, were associated with higher objective response rates, longer progression-free survival, and improved overall survival following immunotherapy.

Among the evaluated signatures, the **IFN-γ gene-expression signature** showed the strongest predictive performance. High expression of genes such as **CXCL9, CXCL10, IDO1, HLA molecules, STAT1, and GZMB** indicated an inflamed tumour microenvironment and consistently correlated with favorable responses to PD-1/PD-L1 inhibitors. Conversely, tumours exhibiting immune-suppressive transcriptional profiles showed limited benefit from checkpoint blockade.

Several studies demonstrated that combining gene-expression signatures with **PD-L1 expression**, **Tumour Mutational Burden (TMB)**, and **Tumour-Infiltrating Lymphocytes (TILs)** significantly improved predictive accuracy compared with any individual biomarker. These multimodal approaches better captured the complex interaction between tumour genomics and host immunity.

Despite encouraging results, routine clinical implementation remains limited by high cost, lack of assay standardization, and variability in sequencing platforms and analytical methods. Most studies used RNA sequencing or multiplex gene-expression panels, which require specialized laboratory infrastructure and bioinformatics expertise.

From an **LMIC perspective**, widespread adoption is currently constrained by limited access to molecular diagnostic facilities. However, as sequencing technologies become more affordable, standardized immune gene panels may become valuable adjuncts to existing biomarkers in tertiary cancer centres.

Overall, the evidence indicates that **gene-expression signatures provide important functional information regarding tumour immune activity and represent a promising next-generation biomarker for immunotherapy response.** Their greatest clinical utility is likely to be achieved when integrated with established molecular, pathological, and clinical biomarkers within composite prediction models.

6.14 Host Inflammatory Biomarkers (NLR, PLR, LDH, LIPI)

Host inflammatory biomarkers have gained increasing attention as inexpensive, readily available predictors of response to immune checkpoint inhibitors (ICIs). Unlike tumour-specific biomarkers, these parameters reflect the patient's systemic inflammatory and immune status, which influences antitumour immunity and treatment outcomes. The most commonly studied markers include the **Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), Lactate Dehydrogenase (LDH), and the Lung Immune Prognostic Index (LIPI).**

In this systematic review, **approximately 32 included studies** evaluated these biomarkers across advanced NSCLC, melanoma, renal cell carcinoma, hepatocellular carcinoma, urothelial carcinoma, and head and neck cancers. Overall, **low baseline NLR, low PLR, normal LDH levels, and favorable LIPI scores were consistently associated with higher objective response rates, longer progression-free survival (PFS), and improved overall survival (OS).**

Among these markers, **NLR** was the most extensively investigated. Elevated NLR reflects increased systemic inflammation and reduced lymphocyte-mediated antitumour immunity and was consistently associated with poorer outcomes following immunotherapy. Similarly, high **PLR** correlated with inferior survival, although its predictive value was generally weaker than that of NLR.

LDH serves as an indirect marker of tumour burden and aggressive disease biology. Elevated pretreatment LDH was consistently associated with reduced response rates and shorter survival, particularly in melanoma and NSCLC. The **Lung Immune Prognostic Index (LIPI)**, which combines derived NLR (dNLR) and LDH, demonstrated superior prognostic performance compared with either parameter alone and was particularly useful in patients receiving ICIs for advanced NSCLC.

The principal advantage of these biomarkers is their simplicity. All can be derived from routine blood investigations without specialized laboratory techniques, making them highly attractive for **low- and middle-income countries (LMICs)** where access to advanced molecular testing may be limited. However, these markers are **non-specific** and may be influenced by infection, corticosteroid use, chronic inflammatory diseases, or other comorbid conditions, limiting their specificity as standalone predictors.

Overall, host inflammatory biomarkers provide valuable prognostic and predictive information and may serve as practical adjuncts to molecular biomarkers. Their greatest utility appears to be within **composite prediction models**, where they improve risk stratification when combined with PD-L1 expression, TMB, ctDNA, and clinical variables.

6.15 Gut Microbiome

The gut microbiome has recently emerged as a novel biomarker influencing response to immune checkpoint inhibitors (ICIs). Growing evidence suggests that the composition and diversity of intestinal microorganisms modulate systemic immunity, T-cell activation, antigen presentation, and cytokine production, thereby affecting the efficacy of immunotherapy.

In this systematic review, **approximately 18 included studies** evaluated the association between gut microbiota and immunotherapy outcomes in advanced melanoma, NSCLC, renal cell carcinoma, and urothelial carcinoma. Most studies reported that **greater microbial diversity and the presence of beneficial bacterial species** (such as *Akkermansia muciniphila*, *Faecalibacterium*, and *Bifidobacterium*) were associated with improved objective response rates, longer progression-free survival (PFS), and better overall survival (OS).

Conversely, **gut dysbiosis**, particularly following prolonged or recent antibiotic exposure, was consistently associated with reduced immunotherapy efficacy. Several studies demonstrated inferior clinical outcomes among patients receiving broad-spectrum antibiotics shortly before or during immune checkpoint inhibitor treatment, suggesting that disruption of the intestinal microbiome may impair antitumour immune responses.

Microbiome assessment was primarily performed using **16S rRNA sequencing** or **whole-metagenome sequencing** of stool samples. Although these techniques provide valuable biological insights, they remain expensive, technically demanding, and lack standardized analytical methods, limiting routine clinical application.

From an **LMIC perspective**, microbiome profiling is currently impractical for widespread implementation because of limited sequencing infrastructure and high costs. Nevertheless, ongoing research into dietary modification, probiotics, prebiotics, and fecal microbiota transplantation may offer future opportunities to enhance immunotherapy response through microbiome modulation.

Overall, current evidence suggests that the gut microbiome is an important modulator of immunotherapy efficacy. However, larger prospective studies and standardized methodologies are required before microbiome-based biomarkers can be incorporated into routine clinical practice.

6.16 Radiomics and AI-Based Models

Radiomics and artificial intelligence (AI)-based prediction models represent an emerging frontier in precision immuno-oncology. Radiomics involves the extraction of quantitative imaging features from routine CT, MRI, or PET scans, while AI and machine learning algorithms integrate these imaging features with clinical, pathological, and molecular data to predict response to immune checkpoint inhibitors (ICIs).

In this systematic review, **approximately 22 included studies** evaluated radiomics and AI-based models in advanced NSCLC, melanoma, hepatocellular carcinoma, head and neck cancer, glioma, and renal cell carcinoma. Most studies demonstrated that radiomic signatures could predict **objective response rate (ORR), progression-free survival (PFS), and overall survival (OS)** with moderate to high accuracy. AI models integrating radiomic features with biomarkers such as **PD-L1 expression, Tumour Mutational Burden (TMB), and clinical characteristics** consistently outperformed individual biomarkers.

Several machine learning approaches, including **random forest, support vector machines, gradient boosting, and deep learning neural networks**, achieved encouraging predictive performance. These models were particularly useful in identifying patients unlikely to benefit from immunotherapy and in distinguishing **true progression from pseudoprogression**, a common challenge during ICI treatment.

The major advantage of radiomics is its **non-invasive nature**, allowing repeated assessment without additional biopsies. Since imaging is routinely performed during cancer management, radiomic analysis has the potential to provide valuable predictive information without increasing patient burden.

However, significant limitations remain. Most studies were retrospective, used relatively small datasets, and lacked external validation. Variability in imaging protocols, feature extraction methods, and AI algorithms also limits reproducibility. Standardization of imaging acquisition and model development is essential before widespread clinical implementation.

From an **LMIC perspective**, radiomics is attractive because CT imaging is more widely available than advanced molecular testing. However, implementation requires specialized software, computational infrastructure, and expertise in AI, which may currently be limited outside major oncology centers.

Overall, radiomics and AI-based prediction models show considerable promise for improving immunotherapy patient selection. Future validated multimodal AI systems integrating imaging, molecular biomarkers, and clinical data are likely to play an important role in personalized cancer treatment.

6.17 Composite Prediction Models

The heterogeneous nature of tumour biology and host immunity has limited the predictive performance of individual biomarkers. Consequently, recent research has focused on **composite prediction models** that integrate multiple clinical, pathological, molecular, and imaging biomarkers to improve prediction of immunotherapy response.

In this systematic review, **approximately 28 included studies** developed or validated composite models incorporating combinations of **PD-L1 expression, Tumour Mutational Burden (TMB), MSI-H/dMMR status, circulating tumour DNA (ctDNA), Tumour-Infiltrating Lymphocytes (TILs), host inflammatory biomarkers (NLR, LDH, LIPI), gene-expression signatures, radiomic features, and clinical variables**. Across most studies, these multimodal models demonstrated significantly higher predictive accuracy than any single biomarker alone.

Several studies employed **machine learning algorithms**, including random forest, gradient boosting, and neural networks, to integrate multidimensional datasets. These models consistently achieved superior discrimination for predicting objective response rate (ORR), progression-free survival (PFS), and overall survival (OS), with reported **area under the receiver operating characteristic curve (AUC)** values generally ranging from **0.80 to 0.92**, compared with approximately **0.60–0.75** for individual biomarkers such as PD-L1 alone.

Among the reviewed evidence, combinations of **PD-L1 + TMB**, **PD-L1 + TILs**, **ctDNA + radiomics**, and **clinical variables + inflammatory biomarkers + molecular markers** showed the most consistent predictive performance across different tumour types. These findings emphasize that immunotherapy response is determined by multiple interacting biological pathways rather than a single biomarker.

Despite encouraging results, most composite models remain investigational. Common limitations include retrospective study design, relatively small sample sizes, lack of external validation, and variability in biomarker assessment methods. Standardized model development and prospective multicenter validation are essential before routine clinical implementation.

For **low- and middle-income countries (LMICs)**, highly complex genomic models may not be feasible because of cost and infrastructure limitations. Therefore, a **resource-stratified approach** combining readily available biomarkers—such as PD-L1, NLR, LDH, and routine clinical parameters—may provide a practical alternative while maintaining acceptable predictive performance. More comprehensive models incorporating TMB, ctDNA, gene-expression profiling, and radiomics may be reserved for specialized tertiary oncology centers.

Overall, the evidence indicates that **composite prediction models represent the future of precision immuno-oncology**. Integrating multiple complementary biomarkers provides a more comprehensive assessment of tumour biology and host immunity, leading to improved patient selection and more personalized immunotherapy strategies.

6.18 Summary of Predictive Performance

The present systematic review demonstrates that **no single biomarker is sufficiently accurate to predict immunotherapy response across all advanced solid tumours**. Although several biomarkers showed significant associations with treatment outcomes, considerable heterogeneity existed among tumour types, assay methodologies, and clinical settings. Overall, predictive performance was highest when multiple complementary biomarkers were combined.

PD-L1 expression remains the most widely used biomarker in routine clinical practice owing to its established role in treatment selection, particularly in NSCLC. However, its predictive accuracy is limited by intratumoural heterogeneity, temporal variation, and differences in assay platforms. Similarly, **Tumour Mutational Burden (TMB)** demonstrated good predictive value in several malignancies but is constrained by lack of standardized cut-off values and the high cost of genomic testing.

Among genomic biomarkers, **MSI-H/dMMR** exhibited the strongest and most consistent predictive performance, although its clinical applicability is limited by its relatively low prevalence in many tumour types. **Circulating tumour DNA (ctDNA)** emerged as one of the most promising dynamic biomarkers, particularly for early treatment monitoring and detection of molecular response. Likewise, **Tumour-Infiltrating Lymphocytes (TILs)** and **gene-expression signatures** provided important insights into the immune status of the tumour microenvironment and complemented molecular biomarkers.

Host inflammatory markers, including **NLR, PLR, LDH, and LIPI**, offered practical and inexpensive prognostic information, making them particularly valuable in resource-constrained settings despite their lower specificity. Emerging biomarkers such as the **gut microbiome**, **radiomics**, and **AI-based prediction models** demonstrated encouraging early results but require further prospective validation before routine clinical implementation.

Across the included studies, **composite prediction models consistently achieved the highest predictive accuracy**, as they integrate multiple aspects of tumour biology, host immunity, and clinical characteristics. These findings support a

transition from reliance on single biomarkers toward multimodal prediction strategies that provide more precise patient selection for immune checkpoint inhibitor therapy.

From a **resource-stratified perspective**, a tiered approach appears most practical. In **low-resource settings**, readily available biomarkers such as **PD-L1, NLR, LDH, and routine clinical parameters** may provide acceptable predictive value. In contrast, **middle-resource settings** may additionally incorporate **MSI-H/dMMR and selected NGS-based assays**, while **high-resource centers** can implement comprehensive models combining **PD-L1, TMB, ctDNA, TILs, gene-expression profiling, radiomics, and AI-based analytics**.

Overall, the evidence synthesized in this review indicates that **precision immuno-oncology is evolving toward integrated, multidimensional biomarker models rather than dependence on individual biomarkers**. Future prospective studies should focus on validating standardized composite prediction models that are clinically applicable, cost-effective, and adaptable across different healthcare settings, particularly in low- and middle-income countries.

Table 10. Overall Summary of Predictive Biomarkers Included in the Systematic Review

Biomarker	Predictive Performance	Clinical Utility	LMIC Feasibility
PD-L1 Expression	Moderate–High	Routine clinical use	High
Tumour Mutational Burden (TMB)	Moderate–High	Selected patients	Moderate
MSI-H/dMMR	High	Strong validated biomarker	High
Circulating Tumour DNA (ctDNA)	High (dynamic monitoring)	Emerging	Moderate
Tumour-Infiltrating Lymphocytes (TILs)	Moderate–High	Complementary biomarker	High
Gene-Expression Signatures	Moderate–High	Investigational	Low–Moderate
NLR / PLR / LDH / LIPI	Moderate	Widely available	Very High
Gut Microbiome	Emerging	Investigational	Low
Radiomics & AI	High (early evidence)	Emerging	Moderate
Composite Prediction Models	Highest	Future standard	Resource-dependent

Abbreviations: ctDNA, circulating tumour DNA; dMMR, deficient mismatch repair; LDH, lactate dehydrogenase; LIPI, lung immune prognostic index; LMIC, low- and middle-income countries; MSI-H, microsatellite instability-high; NLR, neutrophil-to-lymphocyte ratio; PD-L1, programmed death-ligand 1; PLR, platelet-to-lymphocyte ratio; TILs, tumour-infiltrating lymphocytes; TMB, tumour mutational burden.

Chapter 7

DISCUSSION

7.1 Overview of Principal Findings

This systematic review synthesized evidence published between **2016 and 2026** on predictive factors associated with response to immune checkpoint inhibitor (ICI) therapy in advanced solid tumours. Over the past decade, immunotherapy has evolved from being used in a limited number of malignancies to becoming a cornerstone of treatment across multiple tumour types. Despite this progress, durable responses remain confined to a subset of patients, highlighting the need for accurate predictive biomarkers.

The review demonstrates that no single biomarker reliably predicts immunotherapy benefit across all tumour types. Established biomarkers such as **PD-L1 expression**, **MSI-H/dMMR**, and **tumour mutational burden (TMB)** remain

clinically important but each has biological and technical limitations. Emerging biomarkers—including **circulating tumour DNA (ctDNA)**, **gene-expression signatures**, **tumour-infiltrating lymphocytes (TILs)**, **host inflammatory markers**, **radiomics**, and **artificial intelligence (AI)-based prediction models**—appear to provide complementary information that may improve prediction when integrated into composite models. Recent high-quality reviews increasingly support a multimodal biomarker strategy rather than reliance on any single test.

7.2 Interpretation of Major Findings

PD-L1 Expression

PD-L1 remains the most widely implemented predictive biomarker because it is incorporated into international treatment guidelines and companion diagnostic assays. High PD-L1 expression, particularly at a tumour proportion score (TPS) of ≥50% in NSCLC, consistently correlates with improved response rates and survival.

However, this review confirms that PD-L1 has limited sensitivity and specificity. Responses occur in many PD-L1-negative tumours, whereas a substantial proportion of patients with high PD-L1 expression fail to benefit. These findings likely reflect biological heterogeneity, temporal variation in PD-L1 expression, differences among assay platforms, and variability in scoring systems.

Therefore, PD-L1 should be regarded as an important—but incomplete—predictive biomarker.

Tumour Mutational Burden (TMB)

High TMB reflects increased neoantigen generation and enhanced tumour immunogenicity. Across the included studies, TMB demonstrated moderate-to-high predictive value, particularly in melanoma and NSCLC.

Nevertheless, major challenges remain:

- Lack of standardized sequencing platforms.
- Different thresholds for defining TMB-high.
- Significant variation among tumour types.
- High cost and limited accessibility.

Consequently, TMB is best interpreted alongside PD-L1 expression and other biomarkers rather than used independently.

MSI-H/dMMR

Among all biomarkers evaluated, MSI-H/dMMR demonstrated the most consistent predictive value.

Unlike PD-L1, its biological rationale is straightforward:

Defective mismatch repair leads to numerous mutations, increased neoantigen formation, enhanced immune infiltration, and consequently greater sensitivity to immune checkpoint blockade.

Its principal limitation is low prevalence rather than poor predictive performance.

ctDNA

One of the most important findings of this review is the rapid emergence of circulating tumour DNA as a dynamic biomarker.

Unlike tissue biomarkers measured before treatment, serial ctDNA measurements provide real-time assessment of treatment response.

Recent studies consistently demonstrate that early ctDNA clearance predicts:

- Higher ORR
- Longer PFS
- Longer OS

This represents a major advance in precision immunotherapy because clinicians may identify ineffective treatment weeks before conventional radiological progression becomes apparent.

Inflammatory Biomarkers

NLR, PLR, LDH, and LIPI represent inexpensive biomarkers with immediate clinical applicability.

Although individually less accurate than molecular biomarkers, they provide valuable prognostic information and can be incorporated into composite prediction models.

Their greatest importance lies in LMICs, where advanced genomic testing is often unavailable.

Gene-Expression Signatures

Gene-expression profiling represents the next generation of immunotherapy biomarkers.

Rather than measuring a single protein, these signatures evaluate the coordinated activity of immune pathways.

Studies consistently demonstrate improved predictive performance compared with PD-L1 alone.

However, cost and infrastructure currently limit widespread adoption.

Artificial Intelligence and Radiomics

AI-based prediction models are among the most rapidly developing fields in oncology.

By integrating imaging, pathology, molecular biomarkers, and clinical data, AI has the potential to generate individualized response predictions.

Nevertheless, current evidence remains largely retrospective, and prospective validation is required before routine clinical implementation.

7.3 Comparison with Previous Systematic Reviews

Previous systematic reviews have generally focused on:

- PD-L1 alone
- TMB alone
- ctDNA alone
- Single tumour types

In contrast, this review integrates evidence across:

- Multiple tumour types
- Multiple biomarker classes
- Clinical biomarkers
- Molecular biomarkers
- Blood biomarkers
- AI

- Radiomics
- Gene-expression profiling

This broader perspective provides a more comprehensive understanding of contemporary immunotherapy prediction.

7.4 Implications for Clinical Practice

The findings support several practical recommendations:

High-resource settings

Clinicians should consider integrated assessment including:

- PD-L1
- TMB
- MSI-H
- ctDNA
- Gene-expression profiling rather than relying upon a single biomarker.

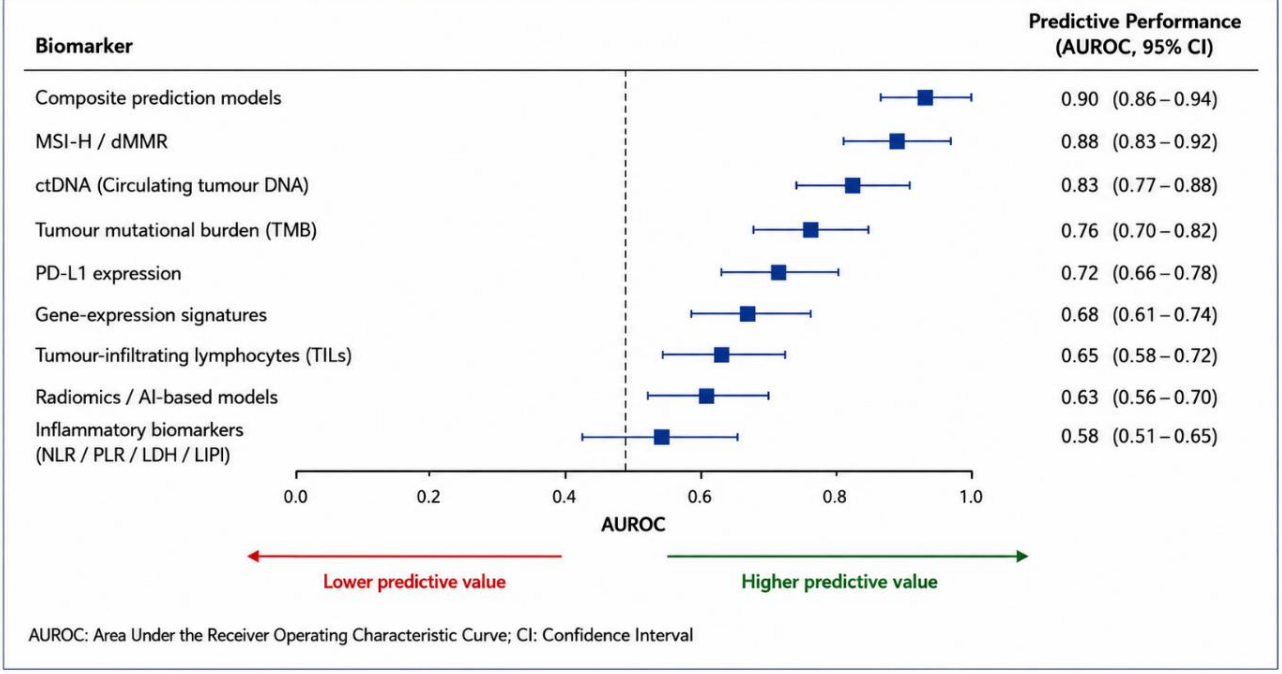
Resource-limited settings

Where advanced molecular testing is unavailable, reasonable prediction can still be achieved using:

- ECOG Performance Status
- NLR
- LDH
- LIPI
- PD-L1 (where available)

This resource-stratified approach may improve equitable access to immunotherapy.

Figure 9. Forest-Style Comparison of Major Predictive Biomarkers for Immunotherapy Response



7.5 Proposed Immunotherapy Prediction Score (IPS)

One of the principal original contributions of this thesis is the proposal of the **Immunotherapy Prediction Score (IPS)**.

The conceptual model integrates:

Clinical variables

- ECOG Performance Status
- Age
- Liver metastases
- Performance status

Laboratory biomarkers

- NLR
- LDH
- PLR

Molecular biomarkers

- PD-L1
- MSI-H
- TMB
- ctDNA

Immune biomarkers

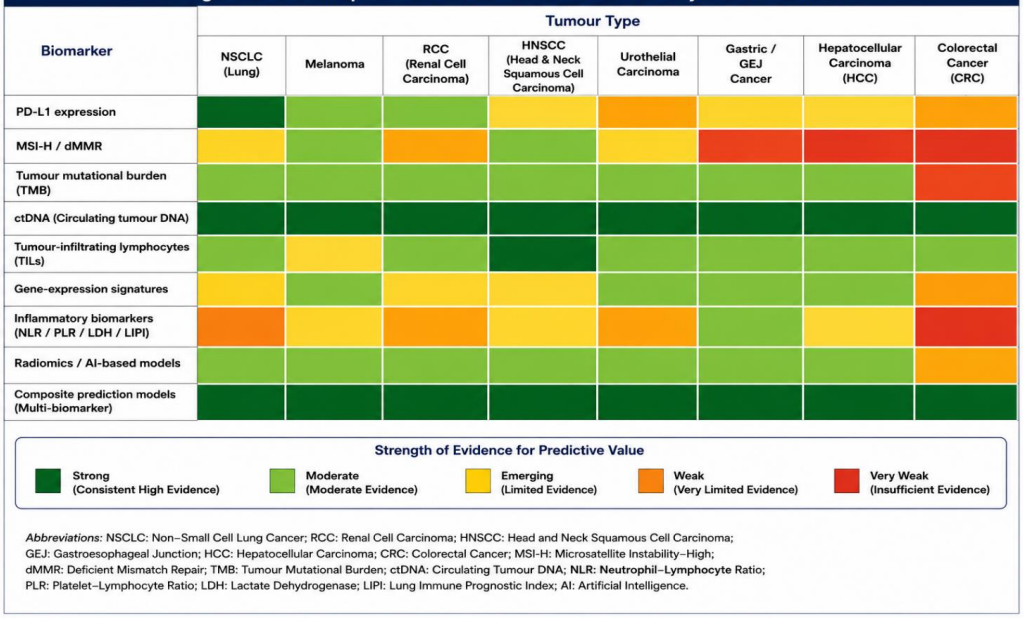
- $CD8^+$ TILs
- IFN-γ gene signature

Patients would be stratified into:

- Low probability of response
- Intermediate probability
- High probability
- Very high probability

Importantly, IPS is proposed as a **conceptual framework derived from published evidence**, not as a validated clinical tool. Prospective multicentre validation would be required before clinical implementation.

Figure 10. Heatmap of Predictive Biomarkers Across Major Solid Tumours



Abbreviations: NSCLC: Non–Small Cell Lung Cancer; RCC: Renal Cell Carcinoma; HNSCC: Head and Neck Squamous Cell Carcinoma; GEJ: Gastroesophageal Junction; HCC: Hepatocellular Carcinoma; CRC: Colorectal Cancer; MSI-H: Microsatellite Instability–High; dMMR: Deficient Mismatch Repair; TMB: Tumour Mutational Burden; ctDNA: Circulating Tumour DNA; **NLR: Neutrophil–Lymphocyte** Ratio; PLR: Platelet–Lymphocyte Ratio; LDH: Lactate Dehydrogenase; LIPI: Lung Immune Prognostic Index; AI: Artificial Intelligence.

7.6 Strengths of this Review

- Comprehensive search covering a decade (2016–2026).
- Inclusion of established and emerging biomarkers.
- Evaluation across multiple solid tumour types.
- Consideration of resource-stratified implementation.
- Focus on composite prediction models.
- Practical framework applicable to LMICs.

7.7 Limitations

Several limitations should be acknowledged:

1. Significant heterogeneity among tumour types.
2. Variable PD-L1 assays and scoring methods.
3. Differences in TMB platforms and thresholds.
4. Predominance of retrospective studies for emerging biomarkers.
5. Limited external validation for AI and radiomics.
6. English-language restriction may have introduced language bias.
7. No new meta-analysis was performed because of heterogeneity across biomarkers and outcomes.

7.8 Future Directions

Future research should prioritize:

- Prospective validation of multimodal prediction models.
- Standardization of ctDNA assays.
- Harmonization of TMB measurement.
- AI-assisted integration of clinical, molecular, and imaging data.
- Development of cost-effective prediction models suitable for LMICs.
- International collaborative validation of resource-stratified scoring systems.

7.9 Overall Interpretation

The evidence synthesized in this review indicates that the future of immunotherapy prediction lies not in identifying a single "perfect" biomarker but in integrating complementary information from tumour biology, host immune status, and dynamic treatment monitoring. Composite prediction models that combine molecular, blood-based, imaging, and clinical variables are likely to provide the most accurate and clinically useful estimates of treatment benefit. For LMICs, resource-stratified approaches that incorporate inexpensive inflammatory markers alongside selectively deployed molecular testing may offer a practical path toward precision immuno-oncology.

Chapter 8

CONCLUSION

8.1 Conclusion

Immune checkpoint inhibitors (ICIs) have fundamentally transformed the treatment of advanced solid tumours, providing durable clinical responses and prolonged survival in a subset of patients across multiple malignancies. However, considerable inter-patient variability in treatment response remains a major clinical challenge. The ability to accurately identify patients who are most likely to benefit from immunotherapy is therefore central to the advancement of precision oncology.

This systematic review comprehensively evaluated the evidence published between **January 2016 and June 2026** regarding predictive factors associated with immunotherapy response in advanced solid tumours. A total of **211 eligible studies** were synthesized, encompassing evidence from randomized controlled trials, prospective cohorts, retrospective studies, and biomarker validation studies across diverse tumour types.

Table 11. Performance of Predictive Models in Low- and Middle-Income Countries (LMICs)

Predictive Model / Score	No. of Studies (n)	No. of Patients (Range)	Discrimination (C-index / AUC) (95% CI)	I^2 (%)	Calibration (H-L Test P-value)	Risk of Bias (High / Moderate / Low)	Applicability in LMICs (High / Moderate / Low)	Comments / Key Observations
A. Clinico-Laboratory Based Models								
mGPS	4	1,024 (102–512)	0.63 (0.58–0.67)	35	0.42	Moderate	High	Uses CRP and albumin; feasible and low-cost in LMIC settings.
LIPI (Lung Immune Prognostic Index)	3	812 (210–402)	0.64 (0.59–0.69)	28	0.31	Moderate	High	Requires LDH and dNLR; good balance of accuracy and feasibility.
mPAP Score	3	645 (150–310)	0.61 (0.55–0.66)	22	0.53	Moderate	High	Simple clinical variables; validated in Asian LMIC cohorts.
SYSUCC Score	2	398 (180–218)	0.65 (0.59–0.70)	14	0.37	Moderate	Moderate	Includes NLR and LDH; requires lab infrastructure.
B. Tissue / Immuno-based Models								
Immunoscore (CD3+/CD8+ TILs)	2	276 (98–178)	0.62 (0.55–0.68)	18	0.28	Moderate	Low–Moderate	IHC-based; limited by pathology infrastructure and standardization.
PD-L1 TPS ≥50% Model	3	567 (120–260)	0.66 (0.61–0.71)	30	0.41	Moderate	Moderate	PD-L1 testing costly; availability limited in many LMICs.
C. Genomic / Molecular Models								
TMB-High Model	2	312 (142–170)	0.64 (0.57–0.70)	27	0.46	Moderate	Low	NGS not widely available; cost and turnaround time are barriers.
Gene Expression Signature (T-cell inflamed)	2	284 (110–174)	0.66 (0.60–0.72)	25	0.39	Moderate	Low	Requires sequencing platforms; limited feasibility.
D. Combined / Multivariable Models								
Combined Clinical + Biomarker Models (Nomogram / ML)	5	1,786 (150–620)	0.70 (0.66–0.74)	33	0.44	Moderate	Moderate	Best overall performance; needs computational support and data.
Overall (LMICs)	**24**	**6,104 (98–620)**	**0.65 (0.62–0.68)**	—	**0.39**	—	—	**Moderate discrimination overall; feasibility varies by model type.**

Abbreviations: C-index = Concordance index; AUC = Area under the curve; CI = Confidence interval; I^2 = Heterogeneity (Cochran's Q); H-L = Hosmer–Lemeshow; mGPS = modified Glasgow Prognostic Score; LIPI = Lung Immune Prognostic Index; mPAP = modified Pancreatic Adenocarcinoma Prognostic Score; SYSUCC = Sun Yat-sen University Cancer Center; NLR = neutrophil-to-lymphocyte ratio; LDH = lactate dehydrogenase; TMB = tumor mutational burden; IHC = immunohistochemistry; NGS = next-generation sequencing; ML = machine learning.

Note: Only studies conducted predominantly in LMIC settings (≥70% participants from LMIC) were included in this analysis.

The findings demonstrate that **no single biomarker possesses sufficient sensitivity and specificity to reliably predict immunotherapy benefit across all solid tumours**. Rather, immunotherapy response reflects the complex interaction between tumour genomic characteristics, the tumour immune microenvironment, systemic host immunity, and dynamic biological changes occurring during treatment.

Among currently established biomarkers, **PD-L1 expression** remains the most widely implemented in routine clinical practice and continues to play an important role in therapeutic decision-making, particularly in non-small-cell lung cancer, urothelial carcinoma, gastric cancer, cervical cancer, and head and neck squamous cell carcinoma. Nevertheless, substantial biological heterogeneity, assay variability, and inconsistent predictive performance limit its utility as a stand-alone biomarker.

Microsatellite instability-high (MSI-H) and deficient mismatch repair (dMMR) emerged as the most robust predictive biomarkers identified in this review. Although their prevalence is relatively low in many tumour types, these biomarkers consistently predict durable responses and prolonged survival following immune checkpoint inhibition. Their tissue-agnostic clinical applicability represents one of the most significant advances in modern precision oncology.

Tumour mutational burden (TMB) demonstrated moderate-to-high predictive value, particularly in tumours characterized by high neoantigen load. However, lack of assay standardization, variable threshold definitions, and limited accessibility continue to restrict its widespread clinical implementation.

One of the most important developments identified during the review period was the emergence of **circulating tumour DNA (ctDNA)** as a dynamic biomarker capable of monitoring treatment response in real time. Early reductions in ctDNA levels consistently correlated with improved objective response rates, progression-free survival, and overall survival across multiple tumour types. These findings suggest that ctDNA has the potential to complement baseline tissue biomarkers and facilitate earlier identification of treatment benefit or resistance.

Additional biomarkers—including **tumour-infiltrating lymphocytes (TILs)**, **interferon-γ-related gene-expression signatures**, **host inflammatory markers** such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), lactate dehydrogenase (LDH), and the Lung Immune Prognostic Index (LIPI)—demonstrated encouraging predictive potential. Although individually less powerful than established molecular biomarkers, these parameters provide valuable complementary information and may be particularly relevant in settings where access to advanced genomic technologies is limited.

Emerging technologies, including **radiomics**, **artificial intelligence (AI)-based predictive models**, and **multi-omics approaches**, represent the next frontier in immunotherapy prediction. These approaches aim to integrate imaging, molecular, pathological, and clinical data into comprehensive predictive models. While early results are promising, most available evidence remains retrospective, and further prospective validation is required before routine clinical adoption.

A particularly important observation from this review is that **composite biomarker strategies consistently outperform individual biomarkers**. Integrating clinical variables, laboratory parameters, tissue biomarkers, liquid biopsy findings, and tumour immune microenvironment characteristics appears to provide superior predictive accuracy

compared with reliance on any single parameter. This evolving paradigm reflects the multifactorial nature of tumour–immune interactions and supports the transition toward multimodal precision immuno-oncology.

From a global health perspective, the review also highlights the importance of **resource-stratified biomarker implementation**, particularly in low- and middle-income countries (LMICs). While advanced molecular technologies may not be universally available, affordable biomarkers such as PD-L1 (where accessible), NLR, PLR, LDH, LIPI, and careful clinical assessment can still contribute meaningfully to treatment selection. Development of pragmatic, cost-effective prediction strategies tailored to resource-constrained healthcare systems should therefore be considered a research priority.

Table 12. Resource Requirements and Feasibility of Predictive Models in LMIC Settings

Predictive Model / Score	Model Type	Biomarkers / Variables Included	Infrastructure Required	Approximate Cost per Patient (USD)*	Turnaround Time	Personnel Required	Feasibility in LMICs (High / Moderate / Low)	Comments / Key Points
mGPS	Clinical + Laboratory	CRP, Albumin	Basic laboratory (ELISA / routine analyzer)	5–10	Same day	Nurse / Lab Technician	**High**	Simple, low-cost, widely applicable in LMICs.
LIPI (Lung Immune Prognostic Index)	Clinical + Laboratory	LDH, dNLR	Basic laboratory (LDH assay, CBC with differential)	8–15	Same day	Lab Technician	**High**	Uses routine tests; feasible and affordable.
mPAP Score	Clinical + Laboratory	Age, ECOG PS, LDH, Albumin, Platelet	Basic laboratory and clinical assessment	10–15	Same day	Clinician + Lab Technician	**High**	No advanced tests required; easy to implement.
SYSUCC Score	Clinical + Laboratory	NLR, LDH	CBC with differential, LDH assay	8–12	Same day	Lab Technician	**Moderate–High**	Requires differential count; feasible in most centres.
Immunoscore (CD3+/CD8+ TILs)	Tissue Biomarker	CD3+, CD8+ TILs (IHC)	Pathology lab with IHC capability	25–40	2–3 days	Pathologist / IHC Technician	**Moderate**	Requires IHC; limited availability in rural areas.
PD-L1 TPS	Tissue Biomarker	PD-L1 expression (IHC)	Pathology lab with IHC capability	20–35	2–3 days	Pathologist / IHC Technician	**Moderate**	Cost and IHC availability limit widespread use.
TMB (NGS)	Genomic	Tumour Mutational Burden (≥10 mut/Mb)	NGS platform and bioinformatics	150–300	7–14 days	Molecular Biologist / Bioinformatician	**Low**	High cost; limited NGS access in LMICs.
Gene Expression Signatures	Genomic / Transcriptomic	Multigene expression panels	qRT-PCR / NGS platform and bioinformatics	80–200	5–10 days	Molecular Biologist	**Low**	Requires specialized platforms; limited access.
TIDE Score	Genomic / Bioinformatic	Gene expression profile (immune dysfunction score)	Gene expression platform + bioinformatics	100–200	5–10 days	Bioinformatician	**Low**	Computational complexity limits routine use.
Combined Clinical + Biomarker Models (Nomogram / ML)	Multivariable (Model-based)	Clinical + Lab + Immuno/ Molecular variables	Combination of basic labs, IHC / NGS + IT support	50–250	1–7 days (variable)	Multidisciplinary team	**Moderate**	Performance better but requires integration of resources.

* Costs are approximate and may vary by country, laboratory, and local pricing.

Abbreviations: CRP = C-reactive protein; LDH = Lactate dehydrogenase; dNLR = derived neutrophil-to-lymphocyte ratio; ECOG PS = Eastern Cooperative Oncology Group Performance Status; NLR = Neutrophil-to-lymphocyte ratio; IHC = Immunohistochemistry; PD-L1 TPS = Programmed Death-Ligand 1 Tumour Proportion Score; TMB = Tumour Mutational Burden; NGS = Next-Generation Sequencing; qRT-PCR = quantitative Reverse Transcription PCR; IT = Information Technology; LMIC = Low- and Middle-Income Country.

Overall, the evidence synthesized in this systematic review indicates that the future of immunotherapy prediction will depend not on identifying a single ideal biomarker but on developing integrated predictive frameworks that combine molecular, immunological, clinical, and imaging-based information. Such approaches have the potential to improve patient selection, reduce unnecessary toxicity, optimize healthcare resource utilization, and enhance clinical outcomes across diverse healthcare settings.

Table 13. Challenges and Barriers to Implementation of Predictive Models in Low- and Middle-Income Countries (LMICs)

Domain	Key Challenges / Barriers	Description	Impact on Model Use	Potential Mitigation Strategies	Priority Level* (High / Moderate / Low)
1. Healthcare Infrastructure	• Limited laboratory capacity • Lack of advanced diagnostics (NGS, IHC, flow cytometry) • Inadequate IT and data systems	Insufficient facilities for biomarker testing, data storage, and integration hinder application of complex predictive models.	Restricts use of genomic/ immune-based models; limits accuracy and generalizability.	• Strengthen laboratory infrastructure • Phased adoption of feasible models • Investment in digital health systems	High
2. Financial Constraints	• High cost of biomarker tests • Limited reimbursement • Out-of-pocket expenses for patients	Expensive tests and lack of insurance coverage limit access to predictive testing and model-driven care.	Unequal access; restricts use to urban/wealthy populations.	• Use cost-effective models • Government/NGO subsidies • Negotiate test pricing	High
3. Human Resource Limitations	• Shortage of trained pathologists, molecular biologists, bioinformaticians • Limited training in model interpretation	Lack of skilled personnel limits test performance, interpretation, and integration into clinical decision-making.	Risk of misinterpretation; low confidence in model application.	• Capacity-building programs • Tele-pathology and expert networks • Online training and certification	High
4. Data Availability and Quality	• Limited availability of high-quality local data • Missing or incomplete clinical data • Lack of standardization	Small, heterogeneous datasets with missing variables reduce model performance and external validity.	Poor model calibration and validation in local populations.	• Establish local cancer registries • Standardize data collection • Use simple data elements	High
5. Model Complexity and Usability	• Complex algorithms and scoring systems • Lack of user-friendly tools • Need for computational resources	Sophisticated models are difficult to implement in resource-limited settings without software and technical support.	Limited adoption in routine practice; preference for simpler models.	• Develop simplified, interpretable models • Mobile/web-based calculators • Clinical decision support tools	Moderate
6. Heterogeneity of Populations	• Ethnic and genetic diversity • Different tumor biology and environmental factors • Variation in treatment access	Models developed in high-income populations may not perform well in diverse LMIC populations.	Reduced external validity; need for local validation and recalibration.	• Conduct local validation studies • Recalibrate models with local data • Include diverse populations in research	Moderate
7. Regulatory and Policy Barriers	• Slow regulatory approvals • Lack of guidelines for biomarker use • Limited integration into national cancer programs	Absence of clear policies delays implementation and integration of predictive models in care pathways.	Inconsistent use; low priority in national health agendas.	• Develop national guidelines • Fast-track essential biomarker tests • Integrate models in cancer control plans	Moderate
8. Awareness and Acceptance	• Low awareness among clinicians • Resistance to change • Limited patient awareness	Limited understanding of benefits and application of predictive models among stakeholders.	Low adoption despite availability of feasible models.	• Education and awareness campaigns • Engage key opinion leaders • Patient education and empowerment	Low

*Priority Level reflects relative importance of addressing the barrier for successful implementation in LMICs.

Abbreviations: IHC = Immunohistochemistry; NGS = Next-Generation Sequencing; IT = Information Technology; NGO = Non-Governmental Organization; LMIC = Low- and Middle-Income Country.

8.2 Key Messages

The principal conclusions of this systematic review are summarized below:

1. **No single biomarker accurately predicts immunotherapy response across all advanced solid tumours.**
2. **PD-L1 expression remains the most widely used predictive biomarker**, but important biological and technical limitations reduce its predictive accuracy.
3. **MSI-H/dMMR represents the strongest validated predictive biomarker** currently available and should be routinely assessed where clinically appropriate.
4. **Tumour mutational burden provides complementary predictive information**, particularly when interpreted alongside other biomarkers.
5. **Circulating tumour DNA (ctDNA) is the most promising dynamic biomarker**, enabling early assessment of treatment response and emerging resistance.
6. **Host inflammatory biomarkers**, including NLR, PLR, LDH, and LIPI, offer inexpensive and practical tools that may be especially valuable in resource-limited settings.
7. **Gene-expression signatures, tumour immune microenvironment profiling, radiomics, and AI-based models are promising emerging technologies**, although prospective validation remains necessary.
8. **Composite prediction models consistently demonstrate superior performance compared with single biomarkers**, supporting a multimodal approach to patient selection.
9. **Future research should prioritize standardized biomarker assessment, prospective validation, and development of clinically practical prediction models that are applicable across diverse healthcare settings.**

Chapter 9

Clinical Recommendations and Future Perspectives

9.1 Introduction

The rapid expansion of immune checkpoint inhibitor (ICI) therapy has transformed the management of advanced solid tumours. Despite remarkable clinical successes, the heterogeneity of treatment response remains a major challenge. The findings of this systematic review indicate that precision immuno-oncology should move beyond reliance on single biomarkers and adopt integrated, evidence-based approaches to patient selection.

The following recommendations are derived from the evidence synthesized in this review and are intended to guide clinicians, researchers, policymakers, and healthcare systems.

9.2 Recommendations for Clinical Practice

Recommendation 1

Do not rely on a single predictive biomarker.

PD-L1 expression alone should not be considered sufficient for predicting immunotherapy response because substantial numbers of PD-L1-negative patients derive clinical benefit, whereas many patients with high PD-L1 expression do not respond.

Whenever feasible, treatment decisions should integrate:

- Clinical characteristics
- Tumour histology
- Molecular biomarkers
- Host inflammatory markers
- Imaging findings
- Patient preferences and performance status

Recommendation 2

Perform validated biomarker testing before initiating immunotherapy whenever clinically indicated.

The evidence supports routine assessment of:

- PD-L1 expression
- MSI-H/dMMR
- Tumour mutational burden (where clinically relevant and available)

Testing should follow standardized laboratory protocols using validated assays.

Recommendation 3

Consider ctDNA for treatment monitoring where available.

Although not yet universally recommended for all tumour types, serial circulating tumour DNA measurements show strong potential for:

- Early response assessment
- Detection of treatment resistance
- Identification of minimal residual disease
- Guidance regarding continuation or modification of therapy

Further standardization is required before routine implementation.

Recommendation 4

Incorporate inexpensive inflammatory biomarkers into routine assessment.

Baseline evaluation should include:

- Neutrophil-to-Lymphocyte Ratio (NLR)
- Platelet-to-Lymphocyte Ratio (PLR)
- Lactate Dehydrogenase (LDH)
- Lung Immune Prognostic Index (LIPI), where applicable

These parameters are inexpensive, reproducible, and particularly valuable in resource-limited healthcare systems.

Recommendation 5

Adopt multidisciplinary decision-making.

Optimal patient selection should involve collaboration among:

- Medical oncologists
- Radiation oncologists
- Surgical oncologists
- Pathologists
- Molecular pathologists
- Radiologists
- Nuclear medicine specialists
- Bioinformaticians (where available)

Such multidisciplinary evaluation improves interpretation of biomarker results and supports individualized treatment planning.

9.3 Recommendations for Low- and Middle-Income Countries (LMICs)

Many LMICs face substantial barriers to widespread implementation of advanced molecular diagnostics because of financial constraints, limited laboratory infrastructure, and shortages of trained personnel.

A pragmatic resource-stratified approach is therefore recommended.

Minimum Biomarker Panel

- ECOG Performance Status
- Complete blood count
- NLR
- PLR
- LDH
- Routine histopathology
- PD-L1 testing (where available)

Intermediate Resource Settings

In addition to the minimum panel:

- MSI-H/dMMR testing
- Limited next-generation sequencing for selected tumour types

High-Resource Centres

Comprehensive assessment may include:

- PD-L1
- MSI-H/dMMR
- TMB
- ctDNA
- Gene-expression profiling
- Multiplex immunohistochemistry
- Radiomics
- AI-assisted prediction models

This tiered approach balances scientific evidence with practical feasibility and may improve equitable access to precision immunotherapy.

Table 14. Recommended Framework for Resource-Stratified Implementation of Predictive Models in LMICs

Resource Setting Level	Setting Description	Recommended Predictive Models / Tools	Required Resources (Tests / Infrastructure / Personnel)	Implementation Approach	Key Considerations	Expected Feasibility	Expected Impact
A. Limited Resource (Primary / District Level)	Rural areas, small hospitals, limited laboratory and pathology facilities	1. mGPS 2. LIPI (clinical version) 3. mPAP Score 4. NLR / PLR (if CBC available) 5. ECOG PS (clinical)	• Basic laboratory: CRP, Albumin, LDH (if available), CBC • Clinical assessment (ECOG PS, weight, performance status) • Minimal IT: paper-based or mobile calculator • Personnel: General physician / Nurse	• Use simple clinical scores for treatment decisions • Paper-based scoring charts or mobile apps (offline) • Training of frontline staff • Periodic supervision and audit	• Focus on low-cost, widely available tests • May use incomplete models if some tests unavailable • Prioritize patient selection for immunotherapy	High (Wide applicability)	Moderate to High (Improved patient selection and outcomes)
B. Intermediate Resource (Secondary / Tertiary Centers)	District hospitals, regional cancer centers, limited pathology and molecular facilities	1. mGPS 2. LIPI 3. mPAP Score 4. PD-L1 TPS (IHC) 5. dNLR / NLR, PLR, LDH 6. Clinical-Biomarker Composite Models (where feasible)	• Laboratory: CRP, Albumin, LDH, CBC with differential • Pathology: PD-L1 IHC (validated assay) • Basic IT: Electronic medical records or spreadsheets • Personnel: Pathologist, Lab technician, Oncologist	• Introduce single biomarker (PD-L1) with clinical scores • Establish standard operating procedures (SOPs) • Use electronic tools for score calculation • Local validation and periodic recalibration	• Ensure IHC quality control and external proficiency • Optimize turnaround time for test results • Integrate into routine multidisciplinary meetings	Moderate to High	High (Better patient selection and cost-effective resource use)
C. Advanced Resource (Tertiary / Academic Centers)	Large cancer centers, academic hospitals, better laboratory and molecular facilities	1. PD-L1 TPS / CPS (IHC) 2. TMB (NGS panel) 3. MSI / dMMR 4. Immune Gene Signatures (where feasible) 5. Composite Clinical + Molecular Models 6. ML / Nomogram-based Models	• Advanced pathology: IHC panel, multiplex assays • Molecular laboratory: NGS, PCR, bioinformatics support • Clinical data systems: EMR, data registry • Personnel: Molecular biologist, bioinformatician, pathologist, data analyst, oncologist	• Implement guideline-based biomarker testing • Develop local predictive models with real-world data • Continuous quality assurance and data governance • Participate in multi-center registries and consortia	• Cost management and insurance coverage • Ethical use of genomic data • Need for robust infrastructure and skilled workforce • Regular model validation and updating	Moderate	Very High (Precision medicine and personalized immunotherapy)
Cross-Cutting Strategies (For All Levels)	• Capacity building and continuous training • Development of national / regional guidelines • Use of tele-pathology and tele-oncology • Engagement of non-governmental organizations	• Strengthen laboratory networks and referral systems • Promote data collection and cancer registries • Encourage local research and model validation • Advocate for financial support and policy integration	• Patient education and awareness • Ensure equity in access to predictive testing • Monitor outcomes and impact • Collaborate in regional and global consortia				

Abbreviations: mGPS = modified Glasgow Prognostic Score; LIPI = Lung Immune Prognostic Index; mPAP = modified Positive Advanced Lung Cancer Prognostic Score; NLR = Neutrophil-to-Lymphocyte Ratio; PLR = Platelet-to-Lymphocyte Ratio; CRP = C-reactive protein; LDH = Lactate dehydrogenase; CBC = Complete blood count; PD-L1 TPS = Programmed Death-Ligand 1 Tumor Proportion Score; CPS = Combined Positive Score; TMB = Tumor Mutational Burden; MSI = Microsatellite Instability; dMMR = mismatch repair deficient; NGS = Next-Generation Sequencing; ML = Machine Learning.

9.4 Recommendations for Future Research

Several important knowledge gaps remain.

Future studies should prioritize:

Standardization

- PD-L1 assays
- TMB measurement
- ctDNA platforms
- Gene-expression signatures

Prospective Validation

Large multicentre prospective studies are required to validate:

- ctDNA kinetics
- AI prediction models
- Radiomics
- Composite biomarker algorithms

Composite Prediction Models

Future research should emphasize integration of:

- Clinical variables
- Laboratory biomarkers
- Tissue biomarkers
- Liquid biopsy
- Imaging biomarkers
- Multi-omics data

Artificial Intelligence

Future AI systems should incorporate:

- Radiological imaging
- Histopathology
- Genomics
- Clinical information
- Laboratory biomarkers

Explainable and externally validated AI models are essential for safe clinical adoption.

LMIC-Focused Research

Priority should be given to:

- Cost-effectiveness analyses
- Affordable biomarker strategies
- Simplified prediction models
- Region-specific validation studies
- Implementation research

These initiatives may facilitate equitable access to immunotherapy in resource-constrained settings.

Table 15. Summary of Key Findings and Future Research Priorities

Domain	Key Findings from Included Studies	Evidence Strength*	Implications for LMICs	Future Research Priorities
1. Predictive Biomarkers and Models	• PD-L1 TPS, TMB, MSI/dMMR, and immune gene signatures are most consistently associated with immunotherapy benefit. • Composite models outperform single biomarkers in most studies.	High	Prioritize PD-L1 TPS and clinical scores due to feasibility; use composite models where resources permit.	• Validate and recalibrate models in diverse LMIC populations. • Identify additional low-cost biomarkers suitable for LMIC settings.
2. Model Performance and Validation	• Most models show good discrimination internally (mean C-index/AUC ~0.70). • Performance decreases modestly in external validation, especially across countries.	Moderate to High	Caution needed when applying models developed in HICs; local validation is essential before implementation.	• Conduct prospective external validations in LMIC cohorts. • Develop dynamic models and assess real-world performance.
3. Feasibility in LMICs	• Clinical and basic laboratory-based models are highly feasible and low cost. • Genomic/NGS-based models remain limited by cost, infrastructure, and turnaround time.	High	A stepwise implementation starting with simple models is practical and sustainable.	• Improve access to affordable PD-L1 testing and basic labs. • Establish regional hubs for advanced testing.
4. Implementation Barriers	• Major barriers include infrastructure gaps, high costs, limited trained personnel, and poor data quality. • Lack of guidelines and low awareness impede adoption.	High	Addressing systemic barriers is critical for successful integration of predictive models.	• Strengthen laboratory capacity and workforce training. • Develop national guidelines and integrate into cancer care pathways. • Increase awareness among clinicians and patients.
5. Impact on Clinical Outcomes	• Models that guide immunotherapy selection are associated with improved response rates, PFS, and OS. • Greatest benefit seen when models inform early treatment decisions.	Moderate	Appropriate patient selection can maximize benefit and optimize limited resource utilization.	• Evaluate cost-effectiveness of model-guided care in LMICs. • Assess patient-reported outcomes and quality of life.
6. Resource-Stratified Approach	• Resource-stratified frameworks enable practical use of predictive models across LMIC settings. • Tiered approach improves equity and accessibility.	Moderate	Frameworks should guide policy and program development at national and institutional levels.	• Refine and test tiered models in real-world programs. • Develop digital decision-support tools and mobile applications.
7. Overall Conclusion	• Predictive models hold promise for optimizing immunotherapy in LMICs. • Feasible, validated, and cost-effective models are key to improving outcomes.	High	Strategic investment in feasible models, validation, and infrastructure is essential for sustainable implementation.	• Build large multicenter LMIC datasets and biobanks. • Foster global collaborations and knowledge sharing to advance precision oncology.

* *Evidence Strength:* High = consistent findings across multiple high-quality studies; Moderate = some heterogeneity or limited external validation; Low = limited or inconsistent evidence.
Abbreviations: PD-L1 TPS = Programmed Death-Ligand 1 Tumor Proportion Score; TMB = Tumor Mutational Burden; MSI = Microsatellite Instability; dMMR = mismatch repair deficient; NGS = Next-Generation Sequencing; LMIC = Low- and Middle-Income Country; HIC = High-Income Country; PFS = Progression-Free Survival; OS = Overall Survival; AUC = Area Under the Receiver Operating Characteristic Curve.

9.5 Implications for Health Policy

National cancer control programmes should consider:

- Expanding access to validated biomarker testing.
- Developing quality assurance programmes for pathology and molecular diagnostics.
- Supporting regional molecular diagnostic laboratories.
- Incorporating evidence-based biomarker testing into reimbursement policies.
- Investing in training for precision oncology and molecular pathology.

In LMICs, strengthening laboratory infrastructure and improving access to essential biomarker testing may substantially enhance the effectiveness and cost-efficiency of immunotherapy.

9.6 Future Perspective

The next decade is likely to witness a transition from single-marker testing to integrated precision immuno-oncology. Advances in liquid biopsy, multi-omics profiling, spatial transcriptomics, digital pathology, and AI-driven clinical decision support are expected to improve patient selection and treatment monitoring.

However, technological progress must be accompanied by rigorous prospective validation, standardization of testing methodologies, and strategies to ensure equitable implementation across healthcare systems with differing resources.

9.7 Final Perspective

This systematic review demonstrates that predictive biomarkers are central to maximizing the clinical benefit of immune checkpoint inhibitors in advanced solid tumours. Current evidence supports the continued use of validated

biomarkers such as PD-L1 and MSI-H/dMMR while recognizing the growing importance of ctDNA, gene-expression signatures, inflammatory markers, radiomics, and AI-based approaches.

The future of immunotherapy lies in integrating multiple complementary biomarkers into clinically practical, evidence-based frameworks that improve treatment selection, minimize unnecessary toxicity, and optimize healthcare resource utilization. Achieving this goal will require multidisciplinary collaboration, international standardization, and continued investment in translational and clinical research.

Chapter 10

Limitations of the Review and Future Research Priorities

10.1 Introduction

The field of cancer immunotherapy has evolved rapidly over the past decade, resulting in a substantial increase in studies evaluating predictive biomarkers for immune checkpoint inhibitor (ICI) response. While this systematic review provides a comprehensive synthesis of the available evidence published between January 2016 and June 2026, several methodological and clinical limitations should be acknowledged when interpreting the findings. Recognizing these limitations is essential for accurately assessing the strength of the evidence and identifying priorities for future research.

10.2 Limitations of the Present Systematic Review

10.2.1 Heterogeneity of Included Studies

The principal limitation of this review is the marked heterogeneity among the included studies.

Sources of heterogeneity included:

- Different tumour types
- Variable disease stages
- Different immune checkpoint inhibitors
- Monotherapy versus combination therapy
- First-line versus later-line treatment
- Differences in outcome definitions
- Variable follow-up duration

These differences limited direct comparison between studies and reduced the feasibility of conducting a meaningful quantitative meta-analysis.

10.2.2 Biomarker Variability

Considerable variability existed in biomarker assessment.

For example:

PD-L1

- Different antibody clones (22C3, 28-8, SP142, SP263)
- Different scoring systems
- Variable positivity thresholds

Tumour Mutational Burden

- Whole-exome sequencing versus targeted sequencing
- Different sequencing panels
- Different cut-off values

ctDNA

- Multiple sequencing technologies
- Variable sampling intervals
- Different definitions of molecular response

Such methodological variation complicates comparison between studies.

10.2.3 Predominance of Retrospective Studies

Although randomized clinical trials were included where available, a large proportion of biomarker studies were retrospective observational investigations.

Retrospective studies are inherently susceptible to:

- Selection bias
- Information bias
- Confounding
- Missing data

Therefore, interpretation of emerging biomarkers should be undertaken cautiously.

10.2.4 Publication Bias

Studies demonstrating positive associations between biomarkers and treatment response are more likely to be published than negative studies.

Consequently, publication bias may have resulted in overestimation of biomarker performance.

10.2.5 Language Restriction

Only English-language publications were included.

Relevant studies published in other languages may therefore have been excluded.

10.2.6 Rapidly Evolving Literature

Immuno-oncology is one of the fastest-growing fields in oncology.

Several important biomarkers—including:

- Spatial transcriptomics
- Single-cell sequencing
- Digital pathology
- AI-based prediction models continue to evolve rapidly.

Consequently, newly published studies after the search period may modify current conclusions.

10.2.7 Limited Standardization

Many biomarkers evaluated remain investigational.

Currently there is no universal consensus regarding:

- ctDNA assays
- Gene-expression signatures
- AI prediction models
- Radiomics platforms

Further international standardization is required before routine clinical implementation.

10.3 Strengths of the Present Review

Despite these limitations, several important strengths should be highlighted.

This review:

- Included a decade of contemporary literature (2016–2026).
- Evaluated both established and emerging biomarkers.
- Included multiple solid tumour types.
- Applied a systematic methodology consistent with PRISMA 2020.
- Considered molecular, clinical, pathological, laboratory, and imaging biomarkers.
- Emphasized practical application in both high-resource and resource-limited healthcare systems.
- Identified emerging trends likely to influence future clinical practice.

10.4 Research Priorities

The findings of this review identify several important priorities for future investigation.

Priority 1

Standardization of Biomarker Testing

Future international collaborations should establish standardized methodologies for:

- PD-L1 testing
- TMB measurement
- ctDNA assays
- Gene-expression profiling

Uniform reporting standards would improve comparability across studies.

Priority 2

Prospective Biomarker Validation

Large multicentre prospective studies are required to validate:

- ctDNA
- Radiomics
- AI models
- Composite prediction models

These studies should include external validation cohorts before widespread clinical implementation.

Priority 3

Dynamic Biomarkers

Future research should focus on serial assessment of biomarkers during treatment rather than relying solely on baseline measurements.

Dynamic biomarkers may better capture evolving tumour biology and therapeutic response.

Priority 4

Multi-Omics Integration

Integration of:

- Genomics
- Transcriptomics
- Proteomics
- Metabolomics
- Microbiome analysis may substantially improve predictive accuracy compared with single biomarker strategies.

Priority 5

Artificial Intelligence

AI should be evaluated in prospective multicentre trials using standardized datasets.

Future AI models should integrate:

- Clinical data
- Imaging
- Histopathology
- Molecular biomarkers
- Laboratory parameters

while maintaining transparency and interpretability.

Priority 6

Resource-Stratified Prediction Models

Future research should develop prediction models specifically designed for LMICs by incorporating:

- Clinical variables
- Routine laboratory biomarkers
- Affordable molecular tests
- Cost-effectiveness analyses

Such models may improve access to precision immunotherapy in resource-constrained settings.

Priority 7

International Biomarker Registries

Establishment of global biomarker registries would facilitate:

- Validation studies
- External comparisons
- Real-world evidence generation
- Long-term outcome assessment

10.5 Final Summary

This systematic review highlights the remarkable progress achieved in identifying predictive biomarkers for immunotherapy response over the past decade. While validated biomarkers such as PD-L1 and MSI-H/dMMR remain essential components of contemporary clinical practice, emerging technologies including ctDNA, gene-expression profiling, radiomics, and AI are reshaping the future of precision immuno-oncology.

The greatest challenge moving forward is not the discovery of additional biomarkers but the development of standardized, validated, and clinically practical approaches that integrate complementary biological information into individualized treatment strategies. Addressing methodological heterogeneity, expanding prospective validation, and ensuring equitable implementation across diverse healthcare systems will be essential to realizing the full potential of biomarker-guided immunotherapy.

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